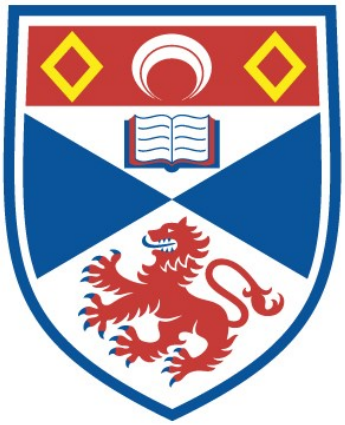
GLYCERIDE STUDIES OF SELECTED SEED OILS

Muhammad Ilyas Qureshi

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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GLYCERIDE STUDIES OF

SELECTED SEED OILS

being a Thesis

presented by

MUHAMMAD ILYAS QURESHI, B.Sc., M.Sc.

to the

UNIVERSITY OF ST. ANDREWS

in application for

THE DEGREE OF DOCTOR OF PHILOSOPHY

March, 1966.

DECLARATION

I hereby declare that the following thesis is based on results of experiments carried out by me, that the thesis is my own composition, and that it has not been presented previously for a Higher Degree.

The research was carried out in the Chemical Research Laboratories of the United College in the University of St. Andrews, under the direction of Dr. F. D. Gunstone.

CERTIFICATE

I hereby certify that Mr. Muhammad Ilyas Qureshi has spent eleven terms at research work under my supervision, has fulfilled the conditions of Ordinance 16 (St. Andrews), and that he is qualified to submit the accompanying thesis in application for the degree of Doctor of Philosophy.

12.4.66

UNIVERSITY CAREER

I entered the Islamia College Lahore, University of the Punjab, in 1951, pursued a recognised course for graduation in Science and graduated B.Sc. in 1955 and M.Sc. in Chemistry (by research on animal fat and seed oil) with upper second class in 1959.

I was admitted as a Research student in the United College of St. Salvator's and St. Leonard, University of St. Andrews, in January 1963.

I was appointed as a Research Assistant in 1959 and as a Research Chemist in 1962, in the West Regional Laboratories, Pakistan Council of Scientific and Industrial Research, Lahore. Whilst there I published, in conjunction with my colleagues four papers in the Pakistan Journal of Scientific Research, one in the Pakistan Journal of Scientific and Industrial Research and one paper in the Journal of the American Oil Chemist's Society.

I am a member of the Pakistan Association for the Advancement of Science.

Publications Based on the Work Reported in this Thesis

1. Determination of the Component Glycerides of Seed Oils containing Saturated, Oleic and Linoleic Acids.
By F. D. Gunstone, F. B. Padley and M. Ilyas Qureshi, Chem. and Ind., 1964, 483.

2. Glyceride Studies, Part II. The Component Glycerides of Seed Oils Containing Saturated, Oleic, and Linoleic Acids.
By F. D. Gunstone, R. J. Hamilton and M. Ilyas Qureshi, J. Chem. Soc., (1965), 47, 319.

3. Glyceride Studies, Part IV. The Component Glycerides of Ten Seed Oils containing Linoleic Acids.
By F. D. Gunstone and M. Ilyas Qureshi, J. Amer. Oil Chemist's Soc., (1965), 42, 961.

4. Glyceride Studies, Part V. The Distribution of Unsaturated Acyl Groups in Vegetable Triglycerides.
By F. D. Gunstone, R. J. Hamilton, F. B. Padley, and M. Ilyas Qureshi, J. Amer. Oil Chemist's Soc., (1965), 42, 966.

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SYNOPSIS

1.

The component glycerides of *J. curcas*, *J. multifida*, *J. gossypifolia* and sunflower seed oils are determined by a new method of crystallisation and column chromatography on silica-silver nitrate.

2.

The component glycerides of nine seed oils (safflower, tobacco, *A. mexicana*, maize, cotton, groundnut, *M. ternifolia*, *G. asiatica* and *M. latifolia*) are determined by thin layer argentation procedure.

3.

The results agree with those obtained by lipolysis or calculated directly from the component acids on the basis of the theory of positional distribution. Moreover, the distribution of oleic, linoleic, hexadec-9 and 11-enoic, lauric and myristic acids in vegetable triglycerides are studied by hydrolysis with pancreatic lipase. The results, discussed in terms of a "selectivity factor", indicate that these unsaturated acids do not compete equally for the secondary hydroxyl group of glycerol.

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1. INTRODUCTION

Attempts have been made to elucidate the composition and structure of natural fats ever since Chevreul showed them to be triesters of glycerol.

The foundation to all modern studies of this problem were laid by Hilditch and his colleagues [12-31] who employed chemical procedures (mainly oxidation) sometimes combined with low temperature crystallisation. Kartha [46,47] modified the Hilditch and Lea oxidation procedure in a manner which enabled him to determine chemically for the first time, the four glyceride categories; U_3, U_2S, US_2, and S_3. Youngs[51] extended this even further by chromatographic separation of oxidised glycerides which he subsequently hydrolysed with pancreatic lipase.

The separation of unmodified glycerides first achieved in part by crystallisation at room temperature and at subzero temperatures, has been modified considerably in recent years. Counter-current distribution [35-40] has been useful, but chromatographic procedures of many kinds [58,59,87-94,107-115] (adsorption and partition chromatography, in columns and on thin layers, with and without added silver nitrate) have been more effective and more extensively employed.

Enzymic hydrolysis [154,158,161-163] by pancreatic lipase offers a complete new approach to this problem.

In short, the methods of analysis of component glycerides have changed rapidly in the last decade and their development will now be reviewed.

2. LOW TEMPERATURE CRYSTALLISATION

The first attempt to analyse triglycerides by crystallisation was made by Holde and Stange[1] who separated a small amount of solid triglycerides from olive oil. Kliment[2,3] isolated oleodistearin and oleodipalmitin from Borneo and stillingia tallows and Bömer[4-6] and his co-workers sometimes carried out hundreds of crystallisations to investigate a single fat. Amberger[7] isolated stearodibehenin from hydrogenated rape oil and the method was extended to cocoa butter by Bauch,[8] Bömer and Engel.[9] Eibner et al.[10] separated the bromo-adducts of linoleodilinolenins and oleodilinoleains and Suzuki and Yokoyama[11] also isolated the brominated glycerides from linseed and soybean oils.

All these earlier investigations were qualitative in character and, although the data served to confirm that fats are largely mixtures of mixed glycerides, no quantitative conclusions were drawn from them.

Hilditch and collaborators[12-14] developed a quantitative method for determining the component glycerides of fat by low temperature crystallisation, Acetone was used as a solvent at temperatures down to -70°C.

It is considered that after a lengthy series of crystallisation and recrystallisation at several temperatures each fraction contains not more than two of the glyceride categories S_3, S_2U, SU_2 and U_3, the first two in the least soluble fractions, the last two in the most soluble fractions and the $S_2U + SU_2$ glycerides in fractions of intermediate solubility.

The fatty acid composition of each fraction is then determined

and the final calculations are made on the assumption that no fraction contains more than two of the main glyceride types. The trisaturated glycerides of individual fractions may be determined by the Hilditch and Lea[42] oxidation method.

Hilditch and his collaborators[15-34] examined many animal and vegetable fats in this way.

Fractional crystallisation is a time consuming procedure and the interpretation of the results involves the assumption described above which does not hold when several unsaturated acids of same chain length are present or when acids of very different chain length are present. This has been demonstrated by the more effective procedures now available such as counter-current distribution[35-40] and thin layer chromatography.[41, 101-103]

3. OXIDATION METHODS

(i) Hilditch

Hilditch and Lea[42] showed that when a fat is oxidized in acetone solution with powdered potassium permanganate, unsaturated glycerides S_2U, SU_2, U_3, are converted into the corresponding azelaoglycerides S_2A, SA_2, A_3, while the completely saturated glycerides S_3 remain unattacked. The acidic azelaic glycerides are separated from the neutral unchanged fully saturated glycerides only with difficulty, because the alkali salts of the azelaoglycerides are strong emulsifying agents. With suitable precautions, however, it is possible to separate the neutral glycerides from the azelaoglycerides. This

method was used by Collin and Hilditch[43] to determine the saturated glycerides S_3 of seed fats rich in saturated acids and from such results Hilditch[44] was led to his rule of "even distribution" in natural seed fats.

(ii) Kartha

Modifications to the Hilditch and Lea procedure have been devised with two ends in mind: there is some hydrolysis of glycerides during oxidation which reduces the accuracy of the method and it would be advantageous if the amount of S_2A, SA_2 and A_3 could also be estimated.

Begemann et al.[45] and Kartha[46,47] showed that hydrolysis was reduced if sufficient acetic acid was added to the reaction mixture to keep it weakly acidic. Kartha demonstrated further that the S_2A fraction could be precipitated as insoluble magnesium salts. With an independent determination of S_3 the four categories of glycerides could be determined.

Kartha used this method to determine the component glycerides of twenty seven natural fats with results different from those obtained by fractional crystallisation. His results agree with values calculated by his rule of restricted random distribution.

Several investigators have compared Kartha's oxidation procedure with other methods. Luddy et al.[48] examined lard, chicken fat, palm and cotton-seed oils by Kartha's oxidation procedure and by fractional crystallisation. Both methods gave similar results except

for palm oil but Eshelman and Hammond[49] consider that neither the Hilditch-Lea[42] method nor the Kartha procedure is very reliable.[46, 47] Lakshiminarayana and Rebello[50] concluded that whilst both oxidation methods give high values due to unoxidized material, the amount is higher in Kartha's[46,47] method. This increase has been shown to be due to the formation of ketols from the unsaturated acids in the Hilditch[42] method and to the corresponding acetyl derivatives in Kartha's[46,47] oxidation.

Kartha's proposed method has the advantage that it requires a considerably smaller quantity of fat than the Hilditch and Lea oxidation procedure. It is limited however to providing information about glycerides in four categories.

(iii) Youngs

Youngs[51] has developed a method for the determination of six glyceride classes S_3, SSU, SUS, SUU, USU and U_3 in fat. The method involves a quantitative oxidation of the fat with periodate and permanganate, similar to that described by Von Rudloff.[52] The oxidized fat is fractionated on a liquid liquid partition column into two fractions, the first contains the unattacked trisaturated glycerides S_3 and monoazelaoglycerides (derived from S_2U) and the second contains di- and tri- azelaoglycerides (derived from SU_2 and U_3). Each fraction is subjected to hydrolysis with pancreatic lipase and the liberated fatty acids are analysed by gas chromatography. The composition of the fractions as well as of the fat is also

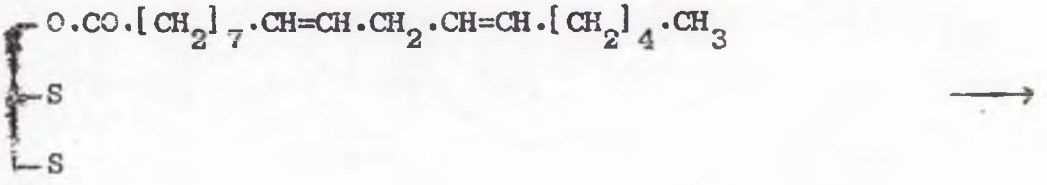
determined by gas chromatography of the derived methyl esters and from the overall results the proportion of the six glyceride classes are calculated.

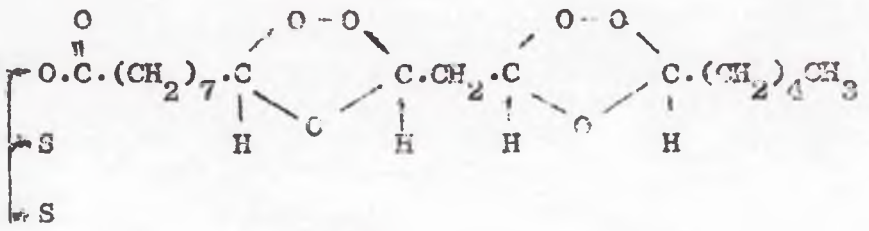
Linseed oil, chicken, rat and pig fats have been examined in this way. The results do not agree with a theory previously proposed by Youngs,[53] but agree well with those calculated by Van der Wal's[54] theory.

All the common unsaturated acids are oxidized to azelaic acid and are therefore indistinguishable. However, the results have demonstrated by confirming Van der Wal's theory that glyceride composition can be calculated from lipolysis results.

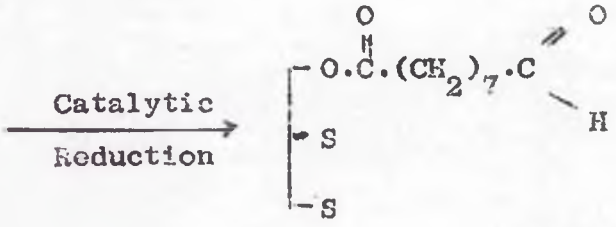
(iv) Ozonolysis

Privett and Blank[55] have described a general micro-procedure for the analysis of triglycerides and lecithins based on a combination of reductive ozonolysis and thin layer chromatography. Triglycerides are converted first to ozonides and then by reaction with hydrogen and Lindlar catalyst to aldehydic glycerides (called by the authors aldehyde "cores") thus





Ozonide



Aldehyde Core

The mixtures both of ozonides and aldehyde "cores" are separated by thin layer chromatography and analysed quantitatively by densitometry. The saturated fatty acids in each of the separated aldehyde "cores" are subsequently analysed by gas liquid chromatography as their methyl esters. Triglyceride composition is finally calculated on the basis of analysis of ozonide classes, aldehyde "cores" and the saturated fatty acid composition of each type of acid cores.

Corn oil, lard, cocoa butter and olive oil have been analysed in this way. This method does not distinguish between isomeric glycerides and is unsatisfactory for glycerides with 5 or more double bonds.

4. COUNTER-CURRENT DISTRIBUTION

In counter-current distribution the glyceride to be fractionated

is subjected to repeated partition between two immiscible liquid phases, in multistage apparatus such as was first described by Craig.[56] The theory and practice have been described by Scholfield.[57]

Using a 200-tube automatic apparatus Dutton, Scholfield[35-40] and collaborators have studied the glycerides structure of linseed oil,[39] soy-bean oil,[36] safflower oil[37] and cocoa butter.[38] A mixture of pentane-hexane furfural and nitroethane is used as a solvent system; this separates into two immiscible phases, and 800 to 1100 transfers are made to achieve the separation.

Weight distribution, iodine number, and fatty acid composition are determined after removal of the solvent from the separated fractions. In the study of cocoa butter[38] using labelled substances, the distribution of radioactivity was also determined.

When plotted against the transfer numbers the distribution data gave a series of interrelated curves, the peaks of which indicated difference in molecular composition. From the data and often with supplementary information obtained otherwise, estimates of the proportions of component triglycerides were calculated. In cocoa butter,[38] values for the specific triglycerides including tripalmitin, dipalmito-monoolein and dilinoleo-monoolein are given.

The values obtained by C.C.D. in general are in agreement with those obtained by other means. However, the method is tedious and separations, in many cases, are still incomplete and for these reasons the method is not widely used.

5. CHROMATOGRAPHY

(a) Column Chromatography

(i) Silica Gel and Alumina

Kaufmann[58] has introduced column chromatography for the separation of triglycerides and since then this has been used frequently. Kaufmann[58,59] used alumina, silica gel and Carnolite 100 as adsorbents. A mixture of tributyrin and tristearin was separated quantitatively on Carnolite 100 using chloroform-methanol as solvents.[30] Kaufmann demonstrated that lower molecular weight triglycerides were adsorbed more strongly on Carnolite 100, and silica gel, than those of higher molecular weight but on alumina the order was reversed.

Trappe[61,62] reported the hydrolysis of ester linkages and isomerisation of double bonds by alumina. Walker and Mills[63] fractionated linseed oil triglycerides on alumina into four classes containing 7,6,5 and 4 double bonds. The separation was extended and in addition to these four fractions, the same workers[64] reported the presence of trilinolein and linoleodilinolenin. A preliminary study of soy-bean and sardine oils was also made by Walker.[65]

Holman et al.,[66] fractionated stillingia oil triglycerides on alumina using three coupled columns. The iodine values, refractive indices and absorption at 2600 Å (due to the decadienoic acid present in this oil) showed that fractions obtained were of different properties but no estimate of glyceride classes was made. More recently, the same workers[67] completely separated the normal

C_{16}/C_{18} triglycerides of stillingia oil from those containing optically glycerides active 2:4-decadienoic acid.

Sahasrabudhe and Chapman[68] were unable to separate a mixture of six triglycerides on silica column but substantial fractionation of olive, linseed, corn and seal oils and horse fat were obtained according to chain length and unsaturation.

More recently, Hirayama[69] fractionated triglycerides as their mercuric acetate adducts on silica. The glycerides S_2U, SU_2 and U_3 were separated using solvent mixtures of increasing polarity and the method was extended to a number of vegetable oils.

(ii) Charcoal

Claesson[70] was unable to separate simple triglycerides on charcoal by displacement analysis but advocated that long chain triglycerides adsorbed in the following order:- tripalmitin trimyristin triolein in benzene, chloroform and ether. Hamilton and Holman[71,72] modified the method and completely separated the triglycerides mixtures of trilaurin, trimyristin and tripalmitin using 0.5% trimyristin in benzene as displacer.

(iii) Factice

An excellent technique of column chromatography using factice, a polymerised soybean oil, as a stationary phase and 5% aqueous acetone as a moving phase was described by Hirsch.[73,74] In addition to the separation of glycerides from a model mixture of tributyrin, trimyristin, and tristearin, the analysis of the linseed

oil was undertaken. Eight fractions were obtained, the first being pure trilinolenin (9 double bonds) and the last having one double bond.

(iv) Thermal Gradient

Magnusson and Hammond[75] separated triglyceride mixtures on an apparatus originally designed by Baker and Williams[76] by an automatic crystallisation in a thermal gradient. The mixture was eluted with acetone as mobile phase from a column which was maintained at higher temperature (25-35°), at the top and lower temperature (0-10°) at the bottom. The apparatus was able to separate the model mixtures, but the separation was limited to glycerides whose fatty acids differed sufficiently in chain length or unsaturation.

Jones and Hammond[77] separated cocoa butter into 43 fractions by this method. Similar fractions were pooled, subsequently converted into methyl esters, and then analysed by gas liquid chromatography. The amount of cocoa butter separated as single glyceride classes SU_2 and S_2U was 85%.

(v) Liquid Liquid Partition

A liquid liquid partition chromatography method to separate triglycerides was developed by Black and Hammond.[78] The solvent was a two phase mixture of acetone, heptane and water and silicone treated celite was used as the support. The effect of various operating variables, such as flow rate, sample size, column length and solvent composition were studied using trilaurin and trimyristin. Triglycerides differing by two carbon atoms or one double bond were separated. Cocoa butter was fractionated and subsequently

determination of fatty acids composition of each fraction indicated that useful glyceride separation could be obtained by this method.

(vi) Silica Silver Nitrate

de Vries[79,80] has described an excellent method for the separation of triglycerides according to their degree of unsaturation and the configuration of their double bonds on columns of silica impregnated with silver nitrate. A mixture of tristearin, 1-oleodipalmitin, 1-stearodiolein and triolein were completely separated from one another as were the cis and trans isomers.

The same worker[80] extended the method to palm oil which was fractionated into tripalmitin, dipalmitolein, dipalmitolinolein, palmitodiolein and mixture of palmitodilinolein and triolein.

(b) Thin Layer Chromatography

The principle of thin layer chromatography was described by Izmailov and Shraiber.[81] The method, later developed by Meinhard and Hall[82] and by Kirchner[83], was used exclusively for separating terpenes. Chemists were generally unfamiliar with it, until Stahl[84,85] developed a convenient apparatus for coating plates and showed its wide applicability.

(i) Silica Gel

Mangold and Malins[86] applied this technique to a number of natural fats and obtained separation of different lipid classes. Kaufmann and co-workers[87-92] have published a series of papers in this field and achieved remarkable separation of synthetic mixtures of triglycerides as well as of various natural fats.

Kaufmann et al, used a partition system in which silica gel layers were impregnated with silicone oil[90] or liquid paraffin.[91]

Temporary impregnation is also carried out with hydrocarbons (undecane or tetradecane) which may be removed after the development by heating the plate in a drying chamber. This proved advantageous when saturated triglycerides are to be detected. Aqueous acetone, or acetone acetonitrile (8:2) containing traces of glacial acetic acid, or acetone-methanol (9:1) are used as developing solvents. The separated components are detected with iodine vapours, aqueous rhodamine, Sudan Black B, 2,7, dichlorofluorescein or phosphomolybdic acid.

The separation of simple saturated triglycerides from tricaprylin to tristearin has been achieved.[87] Critical pairs, such as triolein and palmitodiolein, are also separated on paraffin impregnated silica plates by the use of a multiple development technique.

Kaufmann et al.[92] have also shown that hydrogenation and bromination on the plates provides additional useful information. Triglycerides are brominated by placing bromine in the mobile phase and hydrogenation is effected by spraying the portion of the plate containing triglycerides with a solution of collidal palladium. The plate is dried, impregnated with undecane and allowed to stand in hydrogen atmosphere for one hour. Natural cocoa butter, a cocoa butter substitute, olive oil, and lard have been examined in this way.

(ii) Gypsum

Kaufmann and Khoe[89] have used gypsum as an adsorbent. The separated components on the plates are detected by chemical

reactions, similar to those used in paper chromatography. In addition to the simple and mixed triglycerides of palmitic, stearic and oleic acid the samples of cocoa butter and olive oil have been examined on those plates.

(iii) Silica Gel Silver Nitrate

Barrett, Dallas and Padley[93,94] have described an excellent technique for the separation of synthetic and natural glycerides on thin layers of silica gel impregnated with silver nitrate. The isomers such as 1 and 2-linoleodistearins and 1 and 2-oleodistearins have also been resolved. de Vries[95] has also separated elaidodistearin from oleodistearin.

Glass plates can either be coated with a slurry of silica gel in 12.5% solution of silver nitrate (Barrett et al)[94] or the layered plates, prepared normally with silica gel, can be sprayed with a saturated solution of methanolic silver nitrate (Morris).[96] Glyceride mixtures are applied as solutions in chloroform along the base line and the plate is developed by the ascending technique with carbon tetrachloride, chloroform and acetic acid (60:40:0.5). The glycerides are visualised under a U.V. lamp after spraying with 0.2% ethanolic solution of dichlorofluorescein. For quantitative analysis the glycerides were estimated by densitometry after spraying with 5% aqueous phosphoric acid and charring at 250°C. Complete analysis of lard, cocoa butter, palm and cotton-seed oils have been obtained.

This method, with slight modifications and improvements, has been used in several laboratories (including ours, see Part II) in the past three years. Solvent systems have been improved, non-destructive methods of quantitative analysis have been introduced, and the separated glycerides have been further examined by enzymatic hydrolysis and by gas liquid chromatography of the triglycerides and the derived methyl esters. Apart from our work[98-100] which will be detailed later, the following glyceride studies have been carried out by thin layer chromatography.

Reference	Method	Oil
Kaufmann and Mukherjee[97]	Photometric determination,	Cottonseed oil
Blank, Verdino and Privett[101]	methyl pentadecanoate added as marker, G.L.C. estimation of derived esters, lipolysis	Lard and corn oil
de vries, Jurriens[95,102-104] Schouten and Kroesen,[141] and Luddy.[105]	Amount determined either by weighing or glycerol estimation, G.L.C. of derived esters, lipolysis	cocoa butter, palm, groundnut, soybean, cotton-seed oils and lard.
Kaufmann and Wessels[106]	Estimated by weighing, G.L.C. of derived esters, lipolysis.	Sunflower seed oil

(c) Paper Chromatography

Mangold, Lamp and Schlenk[107] separated trilaurin, trimyristin, tripalmitin and tristearin on paper impregnated with silicone oil. Priori[108] separated vegetable oil triglycerides on liquid paraffin impregnated paper and detected 5% rapeseed oil in olive, sesame and arachis oils.

Kaufmann and co-workers[109-115] have described a number of methods of separating synthetic and natural triglycerides by reverse phase partition paper chromatography. The paper is impregnated either temporarily or permanently with a non-polar stationary phase. Liquid paraffin or silicone oil is used for permanent impregnation[109,113] and temporary impregnation is carried out with undecane or tetradecane.[112] Acetone-acetonitrile (8:2), containing traces of glacial acetic acid, or methanol-acetone are used as developing solvents.[110] The best results are obtained when liquid paraffin is used as stationary phase and the chromatograms are developed either by ascending technique or by circular paper chromatography.[115] In circular paper chromatography relatively large amounts of substance (40 mg.) are separated on one chromatogram, which makes it possible to detect minor components which may be present in the total glycerides. The separations are improved considerably when the same chromatogram is developed a number of times.

The unsaturated glycerides on paraffin-impregnated paper are detected with iodine vapour, but no satisfactory method for detecting saturated glycerides has been described. However, after removing the temporary

impregnation the saturated triglycerides are detected with dichlorofluorescein, Rhodamine B, or Sudan Black.[112]

The triglycerides containing short chain or unsaturated fatty acids have higher R_F values in reverse phase system than those with longer chain length or saturated fatty acids. The influence of cis-trans configuration of double bonds on the R_F value is slight.[117] The relation between molecular weight and R_F value has been given in the form of a "Paper chromatographic index".[112]

Twelve mixed and simple synthetic triglycerides from tricaprin to trimyristin were separated either on silicone oil or paraffin impregnated papers and method was further extended to a number of natural fats.[110] Lard, olive oil and cocoa butter were resolved into three components and butter fat gave ten. Soybean oil triglycerides, before and after interesterification, were also examined by Kaufmann and Schnurbusch.[109] Liquid paraffin or silicone oil was used as the stationary phase and acetone-acetonitrile as the mobile phase. Soybean oil was resolved into five glycerides. A remarkable difference between the glycerides of natural soybean oil and interesterified soybean oil was observed.

The separated glycerides were saponified on paper and the free fatty acids liberated with HCl were detected as ferrocyanide complexes and estimated photometrically.[114,118] The glycerides of corn, soybean, and cottonseed oils[112] were also separated, extracted from the chromatogram, and their fatty acids determined quantitatively by paper chromatography.

Kaufmann and Ahmad[119] have discussed the errors involved in

quantitative determination of triglycerides by paper chromatography.

Stainer and Bonar[120] separated the monounsaturated glycerides of cocoa butter and estimated them by comparing the areas of the spots.

Triglyceride separations on paper impregnated with silicone oil or paraffin hydrocarbons were also achieved by Hirayama and Inouye.[121] The separated glycerides were detected with aqueous potassium permanganate and estimated by densitometry. The mercuric acetate adducts of unsaturated glycerides were also separated by circular paper chromatography by Noda and Hirayama.[122]

Vereshchaglin and Skvortsova[123,124] have used partition and silver nitrate chromatography for the separation of cottonseed oil triglycerides. The triglycerides were separated using dodecane as the stationary phase and methanol saturated with silver nitrate and dodecane, as mobile phase. In this way, thirteen glycerides were estimated, including stereodiolein and palmitodiolein.

(d) Gas Liquid Chromatography

Some preliminary investigations have shown that it is entirely feasible to analyse glycerides by means of high temperature gas chromatography. Thus McInnes et al.[125] converted monoglycerides to allyl esters of their corresponding fatty acids and separated them on Apiezon column operated at 241°C. Acetylated mono and diglyceride mixtures were analysed on a silicone grease column operated under isothermal conditions - by Huebner.[126] Fryer, Ormand and Crump[127] separated the triglyceride mixtures from trimyristin to tristearin on silicone columns and fingerprint chromatograms of various natural

fats and oils were obtained. Pelick et al.,[128] separated the triglycerides on a silicone rubber column and demonstrated from the peak areas that 90% of triglycerides were eluted from the column.

Huebner[129] separated a mixture of simple saturated triglycerides from triacetin to tristearin on a temperature programmed silicone rubber column between 240 and 400°C. A thermal conductivity detector responded equally to all glycerides and good agreement between peak areas and molar composition was obtained. The method was extended to lard and butter oil. In butter oil about 12 peaks containing from 26 to 52 carbon atoms were obtained.

Kuksis, McCarthy et al.,[130] have separated some synthetic and natural triglycerides according to carbon number (i.e. the total number of carbon atoms in the three acyl chains) on a stainless steel column packed with 60–50 mesh Chromosorb W coated with 2.25% silicone rubber gum. Complete and apparently quantitative separations of the simple trioctanoin to tristearin were achieved within 40 minutes in the temperature range of 200–320°C. Although loss of the higher molecular weight triglycerides were occasionally encountered because of incomplete volatilization, there was no evidence of a fragmentation of the triglyceride molecules at the moment of volatilization. Coconut, safflower, cottonseed, corn and peanut oils and butter fat have been examined in this way. The triglycerides of oils which contained palmitic, stearic, oleic and linoleic acids separated into only 3 to 6 peaks but in the case of lauric and myristic acids triglycerides about 14 to 15

peaks were obtained. It has been demonstrated that the recovery obtained for the common vegetable oils and butter fat are of the order of 95 to 100% of theoretical carbon yield.

Kuksis[131,(b)] and Huebner[131(a)] have discovered the various problems involved in quantitative analysis of triglycerides by gas liquid chromatography.

Very recently Kuksis and Breckenridge[132] have achieved preparative and analytical separations of butter oil and its molecular distillates. The glycerides of uniform molecular weight were separated in milligram quantities and from their fatty acids composition the triglycerides structure were determined. Because of multiplicity of saturated fatty acids of varying chain length the exact structure could not be assigned.

Litchfield and Harlow[133] have determined the optimum conditions required for gas liquid chromatography of triglycerides. With optimum operating conditions, higher molecular weight triglycerides up to triarachidin are quantitatively analysed. Quantitative gas liquid chromatography of the triglycerides of cocoa butter and other natural fats are discussed. Harlow, Litchfield et al.,[134] have also separated the triglycerides of four eruic acid containing oils. The average fatty acid chain length calculated from the triglycerides composition of each oil agreed closely with that determined by gas liquid chromatography of its fatty acids methyl esters.

The above methods of glyceride analysis by gas liquid chromatography do

not distinguish unsaturated glycerides from the saturated ones having the same carbon number. Youngs and Subbaram[135] and McCarthy and Kuksis[136] have oxidized natural mixtures thus converting unsaturated glycerides into azelaoglycerides with smaller carbon numbers. Youngs et al.,[135,137] separated the saturated and oxidized triglycerides (after methylation of free carbonyl groups) by gas liquid chromatography, on Anakrome ABS (60-70 mesh) coated with 2% SE 30. The flame ionization detector was used and the column was programmed from 260 to 325°C at 3°/min., after which it was run isothermally till all the glycerides were eluted. The isomeric glycerides were also determined by lipolysis of fractionated azelaoglycerides.[51,135] A number of animal and vegetable fats have been examined[137] and the results agreed with those calculated from lipase hydrolysis data according to method of Vander Wal[54] with the exception of human fat and Momordica charantia.[138] The method gives the distribution of individual saturated acids within the glycerides but does not distinguish between unsaturated acids.

Very recently, Subbaram and Youngs[139] have described another method which gives the distribution of both saturated and unsaturated acids. The triglycerides were separated on the basis of total unsaturation on a silicic acid silver nitrate column. The separated glycerides were then oxidized and the composition of each fraction was determined by gas liquid chromatography.[135] Using this method lard and cocoa butter were analysed and 24 and 18 glycerides obtained respectively. This approach permits the

quantitative determination of all the chemically different glycerides of myristic, palmitic, stearic, oleic, linoleic and linolenic acids in fats.

Litchfield, and Farquhar et al.,[140] separated the Cuphea ilavia triglycerides on silica impregnated with silver nitrate by preparative thin layer chromatography and each fraction was further examined by gas liquid chromatography without any prior modification. The fat, containing high proportion of decanoic acid(95%) was resolved into 17 components. Jurriens and Kroesen[141] also analysed the triglycerides of cocoa butter, sumatra palm oil and lard. The triglycerides were separated by thin layer chromatography and fractions were further examined by gas liquid chromatography after hydrogenation in order to determine the distribution of saturated acids. In this way, in addition to individual triglycerides, the positional isomers were also determined.

6. ENZYMIC HYDROLYSIS

(i) Pancreatic Lipase

Artam and Reale[142] reported that pancreatic lipase effects the partial hydrolysis of triglycerides and this was confirmed by Frazer and Sammons.[143] Mattson and Benedict,[144] from results obtained by feeding rats 2-oleodipalmitin, suggested that digestion proceeded via the 1,2-diglycerides to 2-monoglyceride. Desmuelle and Constantin[145] demonstrated that monoglyceride was formed during digestion of olive oil by rats.

Mattson and Beck[150] extended their studies to the hydrolysis of 2-oleodipalmitin, 2-oleodistearin, 2-palmitodiolein and 1-oleodipalmitin and concluded that the course of the hydrolysis was position specific regardless of the fatty acids in the triglycerides. Borgstrom[146,147] and Schonheyder[148] et al. also concluded that pancreatic lipase hydrolysed the 1 and 3 positions of triglycerides. Further evidence for the positional specificity of pancreatic lipase was furnished by Savary and Desnuelle,[149] who reported that degree of unsaturation and chain length did not influence the rate of lipolysis.

More recently, Alford, David et al.,[151] have shown that lipases from S. aureus and A. flavus hydrolyze the acids attached to the $C_{(2)}$ position of glycerol at about the same rate as acids attached to the $C_{(1)}$ position but the lipase from G. candidum has a high degree of specificity for unsaturated fatty acids, regardless of the position of the fatty acids in the molecules.

Mattson and Beck,[152] and Savary and Desnuelle,[153] have suggested the use of pancreatic lipase for the investigation of fatty acids distribution in natural fats. The procedures for hydrolysing natural fats by pancreatic lipase have been described by a number of workers.[154-157]

Youngs[158] has methylated the free fatty acids of hydrolysed fat with diazomethane and subsequently the whole mixture is injected into a gas chromatographic column and in this way the liberated

acids are analysed. However, Mattson and Volpenhein[158] and Coleman[159] have shown that the degree of hydrolysis affects the composition of the liberated fatty acids. Coleman and Fulton[160] have further suggested that appreciable amount of glycerol is produced during hydrolysis and the composition of fatty acids liberated is not necessarily that of the acids occupying the 1 and 3 positions of the original triglycerides. It has therefore been suggested that isolation and analysis of 2-monoglycerides is more reliable than the analysis of free fatty acids and this procedure is now in common use.

A semi-micro technique for rapid lipase hydrolysis of triglycerides has been described by Luddy,[105] who separated the hydrolytic products by thin layer chromatography and analysed the methyl esters of the liberated acids, the mono, di and triglycerides by gas liquid chromatography.

Savary and Desnuelle[154] examined a number of natural fats by pancreatic hydrolysis and concluded that fatty acid distribution is not random, but that 1 and 3 positions are largely occupied by saturated acids except in pig fat where the 2 position is occupied largely with saturated acids. The unique character of pig fat has been confirmed by Mattson,[161] and Savary and Desnuelle,[162] who also reported that in other animal fats the unsaturated acids occupy the $C_{(2)}$ position. Volpenhein and Lutton[163] have extended their investigations to animal fats with an even number of toes and concluded that palmitic acid occupies the $C_{(2)}$ position in the

triglycerides of wild boar. In other members of this group of animals, the palmitic acid is either randomly distributed among all three positions or the 1 and 3 positions contained more of this acid than the 2 position.

From a study of 48 vegetable seed oils, Mattson and Volpenhein[164,165] concluded that the saturated acids and C_{20} and C_{22} unsaturated acids are esterified exclusively at the primary hydroxyl groups and the secondary hydroxyl group contains C_{18} unsaturated acids predominantly.

The lipase hydrolysis technique has been used by a number of workers, thus Youngs et al.,[135,137] have determined the proportion of the fatty acids in the 1,3 positions and 2 position of their fractionated oxidised triglycerides. Results calculated from lipase hydrolysis data agree with those derived from Youngs oxidation procedure except in the case of Momordica charantia[138] seed oil.

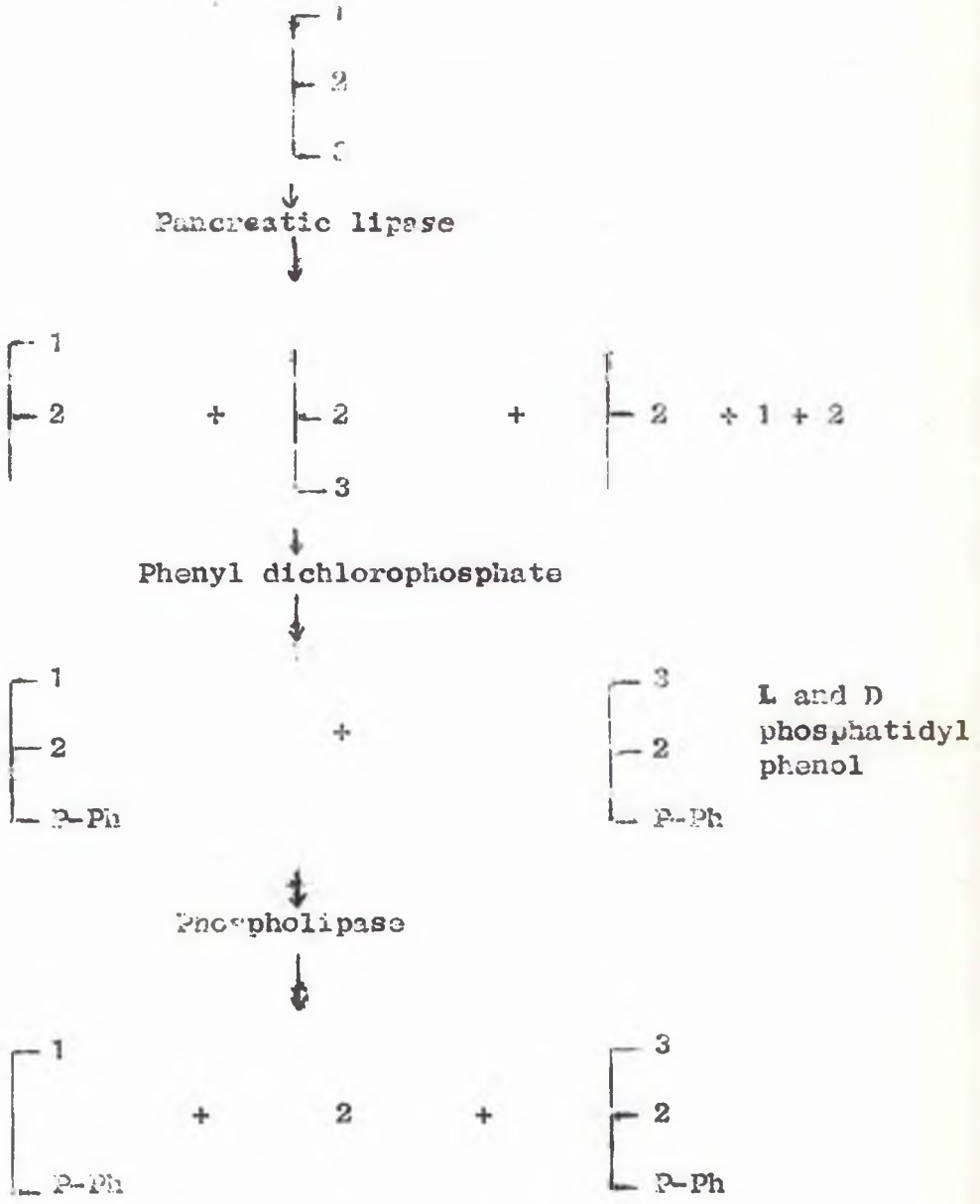
Jurriens and Kroesen[141] have fractionated a number of seed oils by thin layer chromatography and subjected each separated fraction to lipolysis. A similar method has recently been described by Blank et al.[101]

Vander Wal[54] and Coleman et al.,[157] have calculated independently the glyceride components of natural fats from lipolysis results assuming that the acids not present at the 2 position are randomly distributed at 1 and 3 positions. A new

theory of acyl group distribution has been proposed by Gunstone[166] which is also based on results obtained by enzymic hydrolysis.

(ii) Stereospecific Analysis of Triglycerides

Lipase hydrolysis does not distinguish the composition of fatty acids in the 1 and 3 positions. Very recently Brockerhoff[167] has described a method for the analysis of fatty acids in each of the three positions of triglycerides. In this method the 1,2- and 2,3- diglycerides isolated from a partial hydrolysis of triglycerides are converted into L and D phosphatides. These are hydrolysed with phospholipase A from snake venom which attacks only the 2 position of L-phosphatide and leaving a lysophosphatide with fatty acid in position 1, and D-phosphatide with fatty acids 2 and 3 as shown in the scheme.



The products are separated on silicic acid plates and the composition of fatty acids at the various positions is determined by gas liquid chromatography of the methyl esters derived from appropriate fractions.

Fraction	Acid attached to position
Triglycerides	1,2,3
2 monoglycerides(from a separate P. lipase)	2
Lysophosphatide L	1
By difference	3

The distribution of fatty acids in two synthetic triglyceride mixtures and corn oil have been determined and fatty acids in positions 1 and 3 in corn oil, were found to be nearly equivalent.

Brockerhoff[168] subsequently improved this procedure by carrying out the initial lipolysis in presence of hexane to obtain a more representative diglyceride fraction. A number of depot fats of mammals, birds, a fresh water fish, lobster and several vegetable oils have been examined in this way.

7. THEORIES OF GLYCERIDE STRUCTURE

(i) Mono-acid Theory

It was first assumed that natural glycerides were mixtures of simple triglycerides such as tripalmitin, tristearin and triolein in varying proportions. This view is now completely discredited.

(ii) Random Distribution

This theory assumes that the component acids are distributed randomly both between the triglycerides and within each triglyceride molecule. The proportion of any triglyceride can

be calculated from the amount of constituent acids present in the whole fat.

Langenecker,[169] Norris and Matil[170] have expressed the opinion that animal fats may conform to a random distribution and Dean[179] has indicated that in larger land animals the fatty acids are united indiscriminately with glycerol molecules. Hilditch[44] however, suggested that random distribution is confined to trisaturated glycerides and that the other types of saturated unsaturated glycerides behave differently. Dutton and Scholfield et al.,[35-40] on the basis of their counter-current-distribution results concluded that linseed oil, soybean oil and safflower oil follow the random pattern of distribution. If there was any doubt before, hydrolysis of natural fats with pancreatic lipase has clearly shown that fatty acids are hardly ever distributed randomly in glyceride molecules.

(iii) Even Distribution

The theory of even or widest distribution was first proposed by Collin and Hilditch[171] and was later elaborated by Hilditch mainly on the basis of the results obtained by low temperature crystallisation. The theory was stated by Hilditch as follows:-

(i) When a given fatty acid A forms about 35% (mol.) or more of the total fatty acids (A + X) in a fat, it will occur at least once, $G(AX_2)$; in practically all the triglyceride molecules of the fat in question.

(ii) If it forms about 35-35% (mol.) of the total fatty acids (A + X), it will occur twice, $G(A_2X)$, in some triglyceride molecules and of course more frequently the higher the proportion of this acid in the total fatty acids.

(iii) If it forms 70% or more of the total, the remaining fatty acids (X) can at most only form mixed glycerides $G(A_2X)$, and the excess of A then, and broadly speaking then only, appears as a simple triglyceride, (GA_3).

(iv) A minor component acid which forms much less than about a third of the total fatty acids (e.g. 15% or less) will not occur more than once in any triglyceride molecule (and, of course, not at all in many of them).

The rule holds approximately for vegetable fats rich in saturated acids, but it is not applicable to the fats containing larger amounts of several unsaturated acids.

(iv) Restricted Random Distribution

Kartha[46,47,172-176] has assumed that in natural fats all possible molecules are formed, in random proportions provided they are fluid in vivo. In fats in which the random proportions of saturated glycerides S_3 cannot all exist as a fluid the excess saturated acids S, and preferentially that of highest molecular weight, will combine with unsaturated acids U to form the more fluid molecules comprising glycerides S_2U and SU_2 in proportion governed by chance. The resultant

non-random distribution is known as "restricted random distribution".

Kartha[46,47] has formulated a rule for calculating the distribution of glyceride types in a natural fat from the content of S_3 and S_2U glycerides determined by Kartha's oxidation[46] method. Twenty-seven natural fats have been examined the results of which agree well with those obtained by restricted random distribution. Kartha[47] has also demonstrated that the glyceride composition of forty-six natural fats obtained by fractional crystallisation do not agree with those calculated by his theory.

Vander Wal[54] has shown the good agreement of the values of the four triglyceride types of kokum butter, pig fat and peanut oil calculated from lipase hydrolysis data and by restricted random distribution. However, the values of symmetrical and unsymmetrical isomers which comprises the S_2U and SU_2 glycerides are greatly different. A similar relationship in cocoa butter has also been pointed out by Vander Wal.

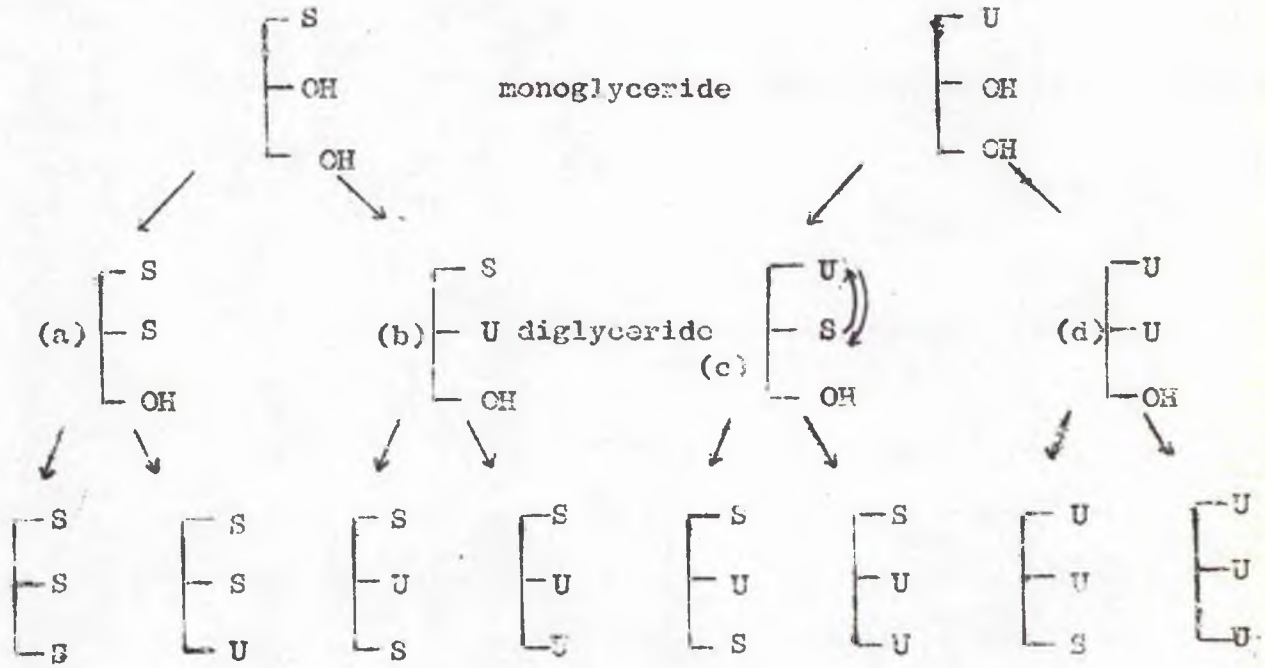
(v) Vander Wal and Coleman and Fulton Theory

Vander Wal[54] and Coleman et al.[157] have developed independently a method of calculating the glyceride structure of natural fats from the pancreatic hydrolysis data. The procedure, in brief, is to calculate, from enzymic hydrolysis data, the percentages in the fat of the four, 1,3 types represented as S-S, U-U, S-U, U-S. The remaining S and U, found

by pancreatic lipase to occupy the 2 positions, is then distributed at random among these categories. The results are the percentage of the six classes (SSS,SUS,SSU,USU,UUS and UUU) .

(vi) Youngs Theory

Youngs[53] has presented a theory for the formation of fats which gives the amounts of various glyceride types equivalent to a random or modified restricted random distribution and at the same time gives a predominance of specific positional isomers. The basis of the theory is a random attachment of fatty acids in the order of 1,2,3 with an intramolecular rearrangement at 1,2 diglyceride level of fat formation. The proposed scheme of fatty acid distribution is given below.



It is also assumed, as confirmed by enzymic hydrolysis studies, that most saturated acids are acylated at 1 and 3 positions and the unsaturated in the 2 position , i.e. the S_2U fraction is predominantly symmetrical and SU_2 predominantly unsymmetrical. The preferred arrangement of the mixed glycerides for this type is 1-saturated 2-unsaturated. The diglyceride having the (b) configuration remains unchanged and that having the opposite configuration (c) rearranges.

Pork fat does not confirm this pattern. It shows predominance of saturated acids at 2 position and of unsaturated acids in the 1 and 3 positions.

The composition of lard, chicken fat, palm oil and cottonseed oil calculated in this way give better agreement with experimental values of Luddy et al.[48] than do either random or restricted random distribution. Youngs[51,137] has now abandoned this theory in favour of that proposed by Vander Wal,[54] because the latter has given good agreement with Youngs[51] oxidation and hydrolysis results.

(vii) Gunstone Theory

This theory which is restricted to vegetable fats depends on the conclusions drawn by Mattson and Volpenhein,[163,164] based on enzymic hydrolysis studies, that the saturated acids are found exclusively at the 1 and 3 positions and as a result of the specific distribution of these acids, the 2 position contained a high proportion of unsaturated acids. Gunstone proposed that

the 2 position is preferentially acylated by oleic, linoleic and linolenic acids and examined three possibilities for the distribution of the remaining acids.

(i) The $C_{(1)}$ and $C_{(3)}$ hydroxyl groups are acylated subsequently by all remaining acids and by C_{18} unsaturated acid not required at $C_{(2)}$.

(ii) One of the primary hydroxyl groups then reacts preferentially with the remaining acids of thistype, and finally the other primary hydroxyl group reacts with the acids that remain.

(iii) One of the primary hydroxyl groups then react preferentially with acids other than unsaturated C_{18} acids, and finally the other primary hydroxyl group reacts with the acids that remain.

In all these cases the distribution of acyl group at each position is statistical.

Gunstone concluded that since there is no means of distinguishing between 1 and 3 positions the theories 2 and 3 give the same arithmetical results. Concerning the two remaining possibilities it was concluded that the theory 1 gives the better agreement between observation and theory. It was shown that fats containing high proportion of C_{18} unsaturated acids would then approximate to the random distribution pattern as found by Scholfield and Dutton et al.[35-40] Gunstone also confirmed by theory 1 the principle of even distribution that fully saturated glycerides are not expected until the content of unsaturated acids falls below 35%.

The views of Kartha[46,47] and Vander Wal[54,157] also fit in the pattern with only slight discrepancies.

(viii) Ordered Distribution Theory[177,178]

The theory assumes that the group of saturated acids are distributed in 1,2 and 3 positions of glycerides in that order by variable degree, and the unoccupied positions are filled by unsaturated acids. By an appropriate adjustment of factors this ordered distribution covers even distribution, random distribution, and a modified form of Youngs distribution.

8. CONCLUSION

Research into the component glycerides of natural fats is now being pursued in many laboratories. New experimental procedures have given new impetus to the devising of theories of acyl group distribution.

When this work was started (1963) there was little or no experimental proof of the distribution theories of Vander Wal, Coleman and Gunstone. The experiments and results to be described were designed mainly to meet this need and also to gain deeper insight into glyceride components of vegetable oils containing unusual unsaturated acids.

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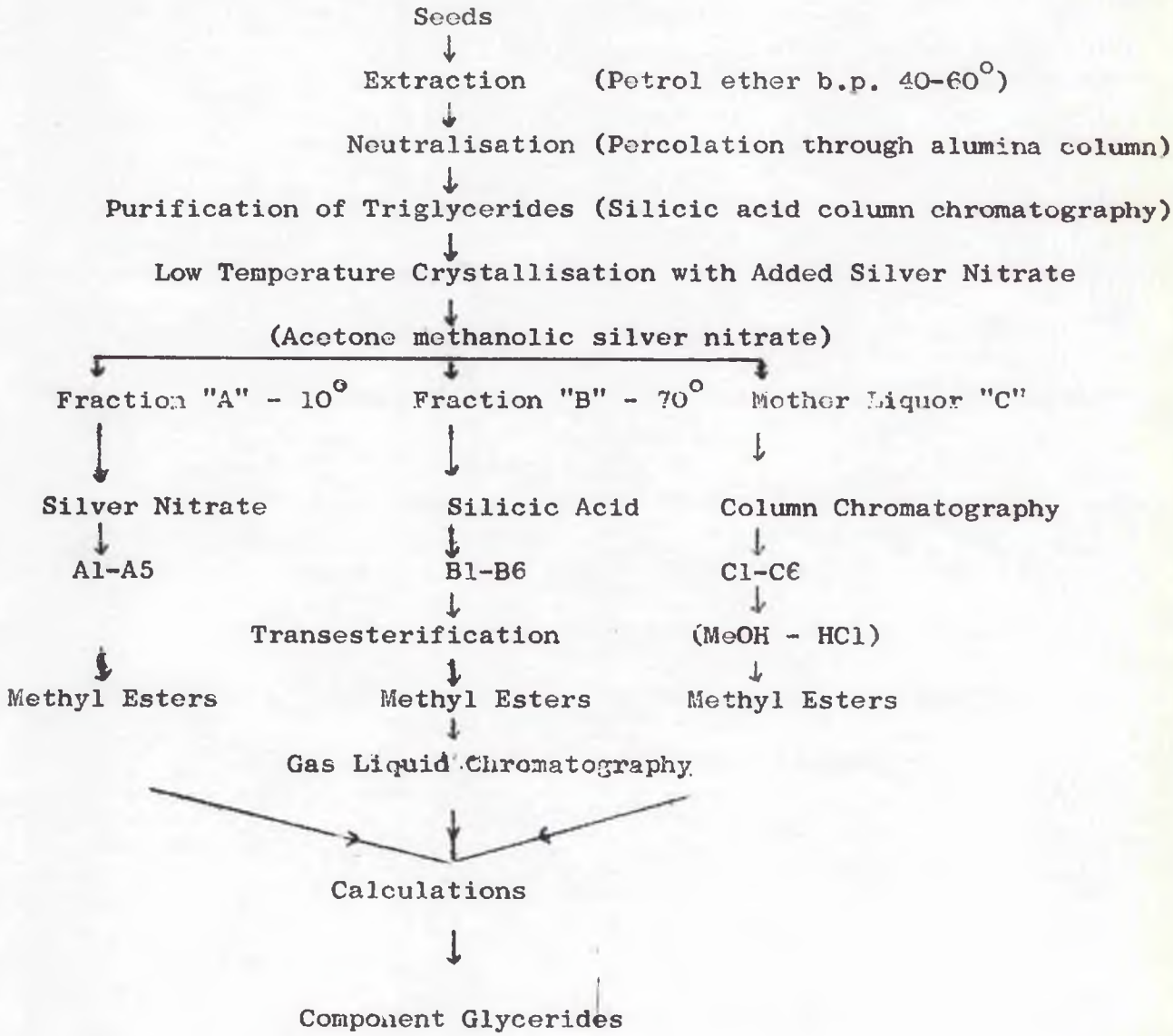
PART I

Determination of Component Glycerides by Low Temperature Crystallisation and Column Chromatography in the Presence of Silver Nitrate.

Scheme of Determining the Component Glycerides



Determination of Component Glycerides by Low Temperature Crystallisation and Column Chromatography in the Presence of Silver Nitrate.

1. DISCUSSION

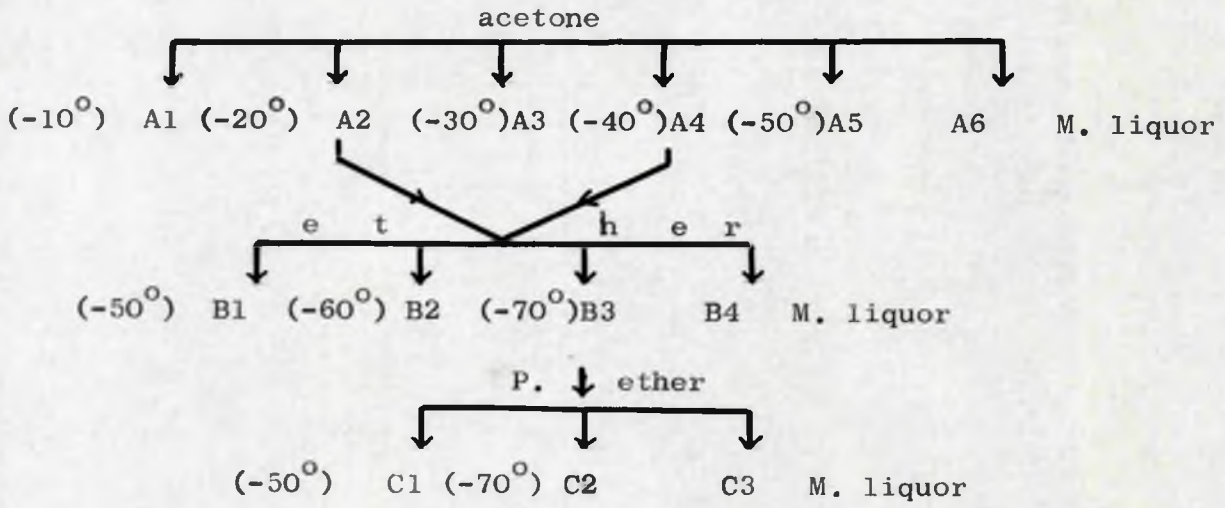
(i) Methods

Component glycerides of Jatropha curcas, J. multifida, J. gossypifolia and sunflower (Nigerian varieties) seed oils have been determined by combination of a new method of crystallisation and column chromatography on silica impregnated with silver nitrate.

Thin layer chromatography of triglycerides on silica impregnated with silver nitrate gave very effective separation of triglycerides according to unsaturation. These oils give nine spots corresponding to trilinolein (222), oleodilinolein (221), saturated dilinoleins (220), dioleolinolein (211), saturated oleolinoleins (210), triolein (111), disaturated linoleins (200), saturated dioleins (110) and disaturated oleins (100) and we have used this technique to monitor the efficacy of separations obtained both by crystallisation and column chromatography.

Hilditch and his associate[1] developed the use of low temperature crystallisation as one of their techniques for studying the component glycerides of a number of animal and vegetable fats. The more recent use of segregation by counter-current distribution between two immiscible solvents has shown that results obtained by crystallisation of the more highly unsaturated seed oils are not entirely satisfactory[2-7] and we have confirmed the incompleteness of these separations by thin layer chromatography.

Low temperature crystallisation of Jatropha curcas seed oil
Solvents:- acetone, ether and P. ether (b.p. 40-60°C)
Silica/silver nitrate adsorbent, benzene ether (9:1)



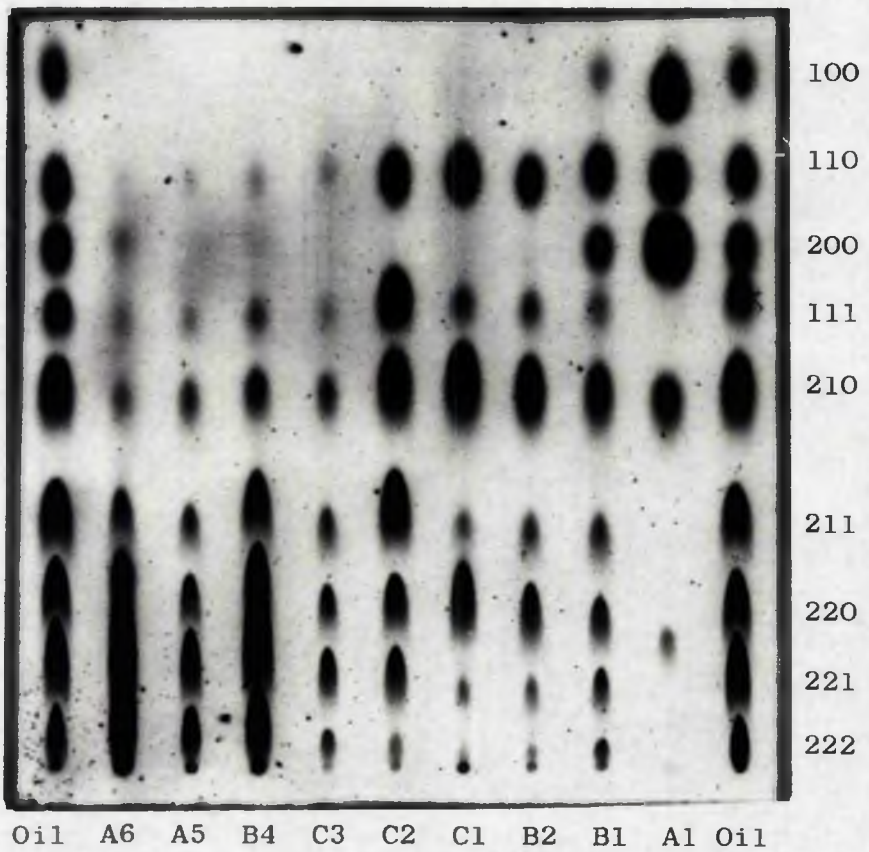


Figure 1

Crushed seeds were thoroughly extracted with boiling petrol ether (b.p. 40-60°) and the extracted oils neutralised by percolation in chloroform through a column of alumina. The triglycerides are subsequently eluted from a column of silica with benzene, more polar solvent removed diglycerides and mono-glycerides.

The neutral triglycerides of J. curcas oil were submitted to a series of crystallisations using ether, acetone, and petrol ether as solvents. Each solution was left at the crystallisation temperature for twenty four hours and separated glycerides fractions were examined by thin layer chromatograpy. (The crystallisation scheme used is given on facing page)

It is quite apparent from the chromatogram that even the least soluble fraction A1 (-10°) contains four glycerides i.e., disaturated oleins (100), saturated dioleins (110), disaturated linoleins (200) and saturated oleolinoleins (210). The fractions of intermediate solubility (B1, B2, C1 and C2) obtained at (-50° and -60°C) contain more or less all the glycerides, except disaturated oleins (100), present in the original oil. The most soluble fractions (C2, C3, B4, A5) and the mother liquors (C3, B4 and A5), though rich in U_2S and U_3 glycerides, are still contaminated with less soluble glycerides.

This incomplete separation by crystallisation may be the result of mutual solubility effects. Presence of unsaturated glycerides [saturated dilinoleins (220)] increase the solubility of saturated glycerides [disaturated oleins (100)]. In addition to mutual solubility and mixed crystal formation, there is another factor which decreases the efficiency of crystallisation. The crystal fractions from component glyceride

mixtures tend to be voluminous and complete separation of crystals from the mother liquor becomes difficult. This problem can be overcome, in part, by recrystallising one or more times. In presence of silver nitrate, however, we have obtained better separations in a single crystallisation.

The separations by low temperature crystallisation in presence of silver nitrate depend upon the ability of compounds having an olefinic linkage to form complexes with silver ions. The complexes formed between the silver ions and a variety of unsaturated hydrocarbons have been investigated extensively by Lucas and Co-workers,[8-10] mainly by partition studies between an organic (carbon tetrachloride) phase and aqueous silver nitrate phase. The prediction of Nichols[11] was varified by Dutton,[12] who separated methyl oleate and methyl linoleate by countercurrent distribution in the system isooctane 0.2M silver nitrate in 90% methanol. de Vries[13,14] also extended this method and separated simple triglyceride mixtures of 3 to 4 double bonds on column of silica impregnated with silver nitrate.

The neutral seed oil triglycerides were crystallised from dilute solutions of acetone and saturated methanolic silver nitrate. (see Table 3, 7, 11, 15 and 19 on pages 69, 73, 77, 81 and 85 respectively). At -10° the precipitate (Fraction A) was mainly disaturated monounsaturated glycerides (S_2U) with some monosaturated glycerides (SU_2) [triunsaturated glycerides (S_3) are absent from these oils]; cooling to -70° gave more crystals (Fraction B) which is mainly SU_2 with a little U_3; the mother liquor was almost entirely U_3.

The efficiency of a single crystallisation in presence of silver

JATROPHA CURCAS SEED OIL

SEPARATION OF GLYCERIDES BY

COLUMN CHROMATOGRAPHY

ON SILICA-SILVER NITRATE.

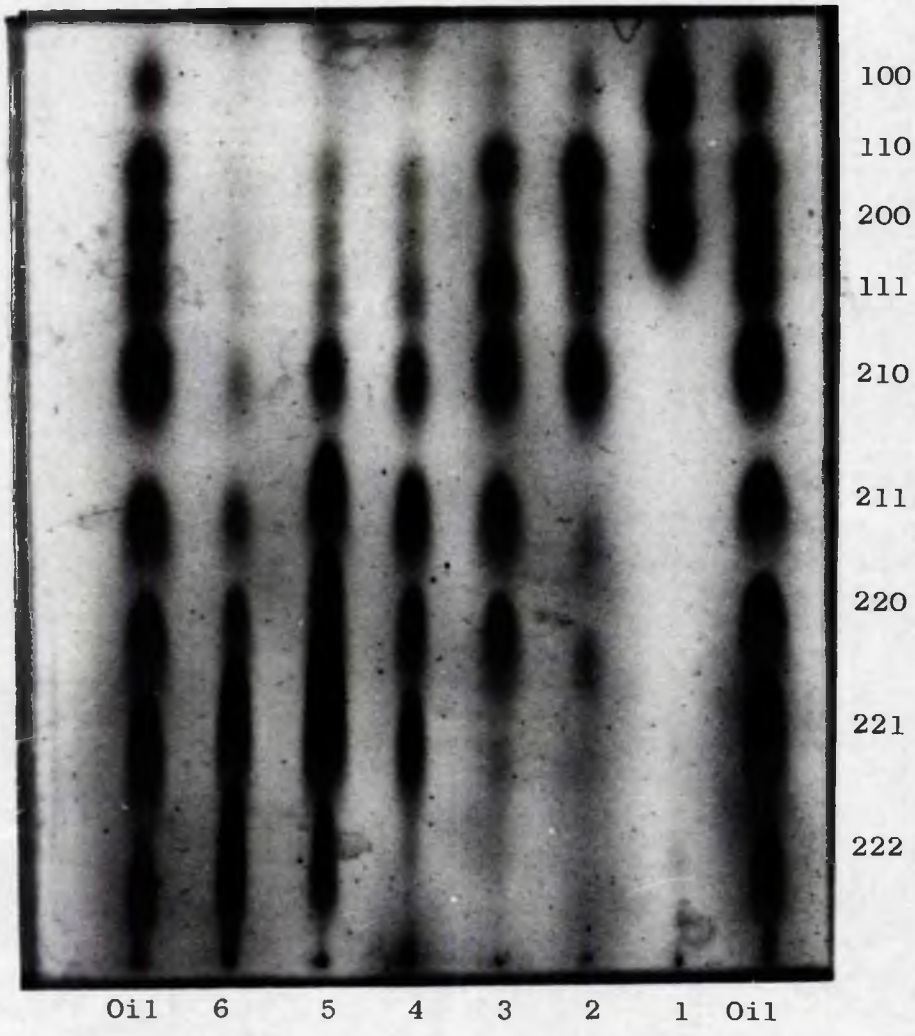


Figure 2

nitrate when compared with ordinary crystallisation is apparent from the chromatograms (see pages 52, 66, 76, 80, 84 and 88) as well as from Table 1 (see page 55).

The A fractions when obtained at -10° contain mainly disaturated oleins (100) and disaturated linoleins (200) (total 70-83% mol.), except in J. curcas oil where the fraction was precipitated at -20°. The B fractions contain mainly saturated dioleins (110), saturated oleolinoleins (210) and saturated dilinoleins (220) (total 72-95% mol.) and C fractions are all rich in triolein (111), dioleolinolein (211), oleodilinolein (221) and trilinolein (222). The glycerides 210 and 111, and 220 and 211, which contain the same number of double bonds are also distinctly separated from one another. The presence of silver ions in the crystallisation solvents appears to increase the differences in solubility between glycerides of varying unsaturation and this leads to improved separation by low temperature crystallisation. Although we did not continue to exploit this procedure we consider that it could be useful in other problems requiring separation of compounds of differing unsaturation.

Attempts to isolate fractions containing not more than two or three different glycerides by column chromatography on silica impregnated with silver nitrate failed with the complex mixture present in the seed oils but were successful with the separated fractions A,B and C. This was demonstrated by thin layer chromatography. (see pages 66, 76, 80, 84 and 88). Our experiment has confirmed, however, the limitations of silica silver nitrate columns in the separation of oils containing oleic and linoleic acids. These separations were improved considerably when simple fractions containing three to four glycerides were submitted to column chromatography.

TABLE. 1

Concentration of glycerides (% mol.) in low temperature crystallisation fractions

Fraction	A			B			C*		
Glyceride	S_2U	SU_2	U_3	S_2U	SU_2	U_3	S_2U	SU_2	U_3
J. curcas	51.7	48.3	-	5.4	94.6	-	-	0.9	99.1
J. multifida	-	-	-	4.8	90.1	5.1	-	8.9	91.1
J. gossypifolia	77.8	22.2	-	2.8	67.6	29.6	-	6.0	94.0
Sunflower (Nigerian)	83.3	16.7	-	-	72.3	27.7	-	3.3	96.7
Sunflower (Nigerian)	69.2	30.8	-	-	89.5	10.5	-	0.8	99.2

*Fraction A insoluble at -10°

Fraction B insoluble at -70°

Fraction C mother liquor

TABLE 2

Concentration (% mol.) of glycerides in sunflower (Nigerian) seed oil fractions obtained by column chromatography.

Fraction	% mol.	100	200	110	210	220	111	211	221	222
B1	3.4			41.2	58.8					
B2	6.7				76.1	13.4		10.5		
B3	6.1				26.2	55.7		18.1		
B4	8.0				21.3	52.5			26.2	
B5	4.1					58.5			41.5	
B6	6.7					38.8			31.3	29.9
C1	12.1				9.1		Tr	89.3	1.6	
C2	7.0					5.8		47.1	47.1	
C3	12.7					3.1		18.9	78.0	
C4	6.0					3.3			95.0	1.7
C5	8.6								77.9	22.1
C6	16.8								42.9	57.1

All the fractions thus obtained contained only one or two glycerides with minor traces of the third glyceride. We recovered the triglycerides from the columns in high yield (97-99%).

The effectiveness of this separation is illustrated in Table 2 (page 58) which shows the concentration of various glycerides in individual fractions. The sunflower oil (Nigerian), which we divided into the largest number of fractions, is selected for this purpose. It is quite apparent that only a few values exceeded 90% mol., otherwise most of the values lie between 50 to 70%. This also demonstrates the inability of the columns to separate unsaturated triglycerides mixture completely from one another.

The seeds oils are divided by column chromatography into 9-12 fractions. After transesterification, the component esters of each fraction are determined by gas liquid chromatography. The sum of the increments from the analysed fractions shows a small loss of linoleic acid and we have adjusted the linoleic acid figures upwards to correct this value. The loss in linoleic acid varies from 1 to 4 units and it is greatest in sunflower oil (Nigerian) and least in J. curcas oil. This loss is directly proportional to the linoleic acid content and can be attributed to incomplete recovery from the silica silver nitrate columns to which this acid adhere very strongly. Similar losses, often more serious, are sometimes encountered in glyceride studies[15]. The composition of each fraction, after adjustment, is then converted from a weight percentage basis to a molar percentage basis. Qualitative evidence of the glycerides present from the thin

layer chromatograms is combined with the quantitative information about the component esters and in almost all cases the component glycerides can be calculated. Fractions which cannot be so calculated are usually so small that reasonable assumptions can be made without introducing serious error into the final results. (see Tables 6, 10, 14, 18 and 22, pages 72,76,80,84 and 88 respectively).

(ii) Results

The results reported in Tables 6, 10, 14, 18 and 22 are discussed, along with results obtained by other methods, on page 140, part III.

(iii) Comments

The method in hand gave very encouraging results and is quite useful, especially when large quantity of the glycerides are required for further studies. The crystallisation in the presence of silver nitrate has proved to be an effective tool for the separation of unsaturated glycerides (or fatty acids from its seed oils) and can be extended to any scale. On the other hand the method is time-consuming, laborious, and gives less satisfactory separation than those now obtainable by the thin layer argentation procedure (Part II).

2. Experimental

(ii) Preparation of Neutral Triglycerides

(a) Extraction

The seeds were separated from the husk and the kernels, after crushing in grinding machine, (Glen Creston, Stanmore, Foreign) were extracted for 14 hours in a soxhlet apparatus with petrol ether (b.p. 40-60°C). The solvent was subsequently distilled off, the last traces under reduced pressure. Residual meal was recrushed and re-extracted with the same solvent. The process was continued till no more oil was found in the petrol ether extract. The extracted oils were stored under nitrogen at 0°.

(b) Neutralisation of the Oil

Activated alumina (12 gm., Peter Spence, Type H, 100-200 mesh) was poured into the column as a slurry with (40 ml.) chloroform. The oil, (8-10 gm.) after dissolving in chloroform, was brought to the column and eluted with a further 700 ml. of this solvent. The solvent was removed and the neutral oil was kept under nitrogen at 0°C.

(c) Detection of Partial Glycerides

Thin layers of kieselgel G (Merck, Darmstadt, according to Stahl)[16] were applied to glass plates (20 cm. x 5 cm.) in a thickness of 250μ by a commercial applicator. The plates, dried and activated by heating at 110°C for half an hour, were stored in an airtight cabinet.

Oil samples (0.01 ml. of a 10% petrol ether solution) were applied to the plates 1 cm. apart on a line about 1-2 cm. from the bottom of the plates. The plates were developed in sealed jars containing

eluting solvent (benzene ether, 3:2) to a depth of 0.5 cm. The jars were lined with solvent-soaked filter papers to give a vapour-saturated atmosphere.

The separated glycerides were subsequently made visible by exposure to iodine vapours or by charring, after spraying with 50% sulphuric acid. Triglycerides appear as large spots near the solvent front; di- and mono-glycerides, if present, form spots lower down the plate.

(d) Separation of Triglycerides from Mono and Diglycerides[17]

Silica gel (30 gm. Whatman, Silica Gel, SG 31) was covered with benzene and after stirring, the slurry was poured into the column (33 x 1.8 cm.). The solution of the oil (1 to 1.5 gm.) in chloroform (5 ml.) was brought to the column and the eluent-flow was adjusted to 1.5-2 ml. per minute and maintained throughout the experiment. The triglycerides were eluted with benzene (200 ml.), diglycerides with benzene-ether (9:1, 200 ml.) and monoglycerides subsequently with ether.

The complete removal of partial glycerides from triglycerides was checked by the thin layer chromatography technique already described and the fractions were rechromatographed, if necessary.

(e) Iodine Value

Iodine values of triglycerides were determined by Wij's method[18].

(ii) Low Temperature Crystallisation with added silver nitrate

Dry acetone was prepared according to Vogel[19]. The acetone was refluxed with successive shaking with small quantity of potassium permanganate and then dried over anhydrous carbonate, filtered, and

distilled.

A saturated solution of methanolic silver nitrate was prepared by refluxing methanol (200 ml.) and powdered silver nitrate (8 gm.) in a flask shielded from light. The solution was cooled to room temperature in a dark place. A fresh solution was prepared for each crystallisation.

A solution of triglycerides (1.5-2.0 gm.) in a saturated solution of methanolic silver nitrate (2.5% containing twice the amount of silver nitrate required to complex with all the olefinic centres) and acetone (3 ml. per 7 ml. of methanolic silver nitrate) was kept in a Dewar flask containing acetone at -10° for 24 hours with occasional shaking, and then quickly filtered through a sintered glass filter cooled to -15°. The precipitate (fraction A) was twice washed with a little cold acetone-methanolic silver nitrate solution kept at -15°. The filtrate was held at -70° for 24 hours and then the precipitate (fraction B) was filtered from the mother liquor (fraction C). The precipitated glycerides were dissolved in hot petrol ether (25 ml.), washed with distilled water (3 x 50 ml.) to remove silver nitrate, and the aqueous washings re-extracted with petrol ether (40 ml.). Solvent was removed from the petrol ether solution after drying over anhydrous sodium sulphate. The mother liquor was concentrated to 20 ml., washed into a separating funnel with petrol ether (50 ml.) and treated as above.

The fractions were kept under nitrogen at 0°.

(iii) Column Chromatography[13,14]

Silver nitrate was deposited on silica gel (Whatman, Silica Gel, SG 31) by suspending the latter (100 gm.) in water containing silver nitrate (33 gm.). The resulting slurry was heated in an oven at 100°C with occasional stirring until most of the water had evaporated and then at 120° for 16 hr. The adsorbent, after cooling to room temperature, was passed through a 60 mesh sieve and kept in an air-tight dark bottle.

A mixture of adsorbent (30 gm. Silica/$AgNO_3$) and pure benzene (150 ml.) was heated to the boil for 5 minutes while stirring. After cooling to room temperature, the slurry was brought into the column (32 x 1.6 cm.) and washed with (50 ml.) benzene. The column was shielded from light.

A solution of each of the triglyceride fractions, (A,B and C) in benzene (5 ml.) was placed on the column and eluted at 1 ml./min. with a range of solvents of increasing polarity (see Tables 4,8,12,16,20) Each eluate was washed with distilled water (3 x 40 ml.) to remove dissolved silver nitrate, dried over anhydrous sodium sulphate, and weighed after removal of solvent. Four to eightfractions were usually collected; these were subsequently examined by thin layer argentation and fractions of similar composition combined.

Thin layer plates were prepared according to Stahl.[16,20] Silica gel G (30 gm. Merck Darmstadt) was thoroughly mixed with water (60 ml.) containing silver nitrate (5 gm.). The resulting slurry was applied to glass plates (20 x 20 cm.) in a thickness of 250μ by a perspex

applicator, especially made for this purpose. The plates were dried and activated by heating at 110° for half an hour immediately before use. Better separation occurred on fresh plates than a plate made a day earlier.

Solutions (2% in petrol ether, 0.5-1.0µl) of each fraction from the column chromatography were applied to the impregnated plates 1 cm. apart on a line 1-2 cm. from the bottom. The plates were developed (40 min.) by ascending elution in air-tight tanks containing benzene-ether (9:1) as eluting solvents. The tanks were lined with solvent soaked filter paper to give a vapour saturated atmosphere in the tanks.

The separated glycerides appeared as black spots when the plate was gently moved in the direction of development under a glass blower's torch.

(iv) Analysis

a. Conversion of Triglycerides to Methyl esters[21,22]

(i) Triglycerides were dissolved in benzene (0.5 ml.) and refluxed with dry methanolic hydrogen chloride (5%, 100% excess) for three hours. After cooling to room temperature, the contents were poured into water (40 ml.) and the methanolic solution was extracted with ether. The ether was washed with 0.5N sodium bicarbonate solution to remove mineral acid and then with water. After drying over anhydrous sodium sulphate, it was evaporated to dryness at 80°C under nitrogen and the methyl esters were examined by gas liquid chromatography.

(ii) Alternatively the triglycerides were refluxed with a solution of sodium (1%) in methanol for five minutes. The mixture, after cooling to room temperature, was poured into cold water (40 ml.) and extracted with hexane. The extract was dried over anhydrous sodium sulphate and after removing the solvent, the methyl esters were examined by gas liquid chromatography.

(b) Gas Liquid Chromatography

The methyl esters of fractions A, B and C and their sub-fractions, obtained by column chromatography, were analysed quantitatively on a Perkin Elmer Fractometer, Model 451 with a flame ionization detector connected to a Honeywell Brown recorder. Standard stainless steel columns ($\frac{1}{4}$"x 20 S.W.G.) of 1 or 2 m. long were packed with a firebrick support (60/80 mesh) coated with either polycthylene glycol succinate or diethylene glycol succinate or Apiezon L. grease as stationary phase. The columns were operated at 185-195°C with nitrogen as carrier gas at a flow rate of 140 ml. per minute. Retention times were measured from the injection of the samples and the results expressed as carbon numbers which were found from a straight line plot of log. V_R^{23} for a range of suitable standards against chain length of saturated acids.

Samples, either as liquid or as petrol ether solutions, were injected with a 10µl Hamilton syringe. All the fractions, except the minor ones, were analysed in duplicate.

The peak areas, except in J. gossypifolia seed oil, were

measured by the triangulation method. The areas were calculated from the peak height multiplied by the width measured at half height. The width was measured accurately from the outer edge of one line to the inner edge of the other line with a 1/100 cm. scale magnifier. In J. gossypifolia seed oil, where the peaks of certain fractions were unsymmetrical, the areas were measured by a planimeter.

3. Calculations and Results

Adjustment of linoleic acid

The values for linoleic acid (increments % wt.) are multiplied by the factor "L" where

$$L = \frac{\text{Amount of the linoleic acid in the original oil}}{\text{Amount of the linoleic acid in the sum of all the fractions}}$$

The values of all the other acids are then adjusted downward by multiplying them by another factor "K" where

$$K = \frac{\text{Amount of the remaining acids in the oil}}{\text{Amount of the remaining acids found in the sum of all the fractions}}$$

After these adjustments the acids are converted to % mol. in the usual way and the component glycerides are calculated.

Calculations for Component Glycerides

It is difficult to make any generalisations regarding the method of calculating the component glycerides as each fraction has to be considered in relation to its component esters and its component glycerides shown qualitatively by thin layer argentation.

JATROPHA CURCAS SEED OIL

SEPARATION OF GLYCERIDES BY LOW TEMPERATURE CRYSTALLISATION (Acetone-Methanolic $AgNO_3$) and BY COLUMN CHROMATOGRAPHY ON SILICA-$AgNO_3$.

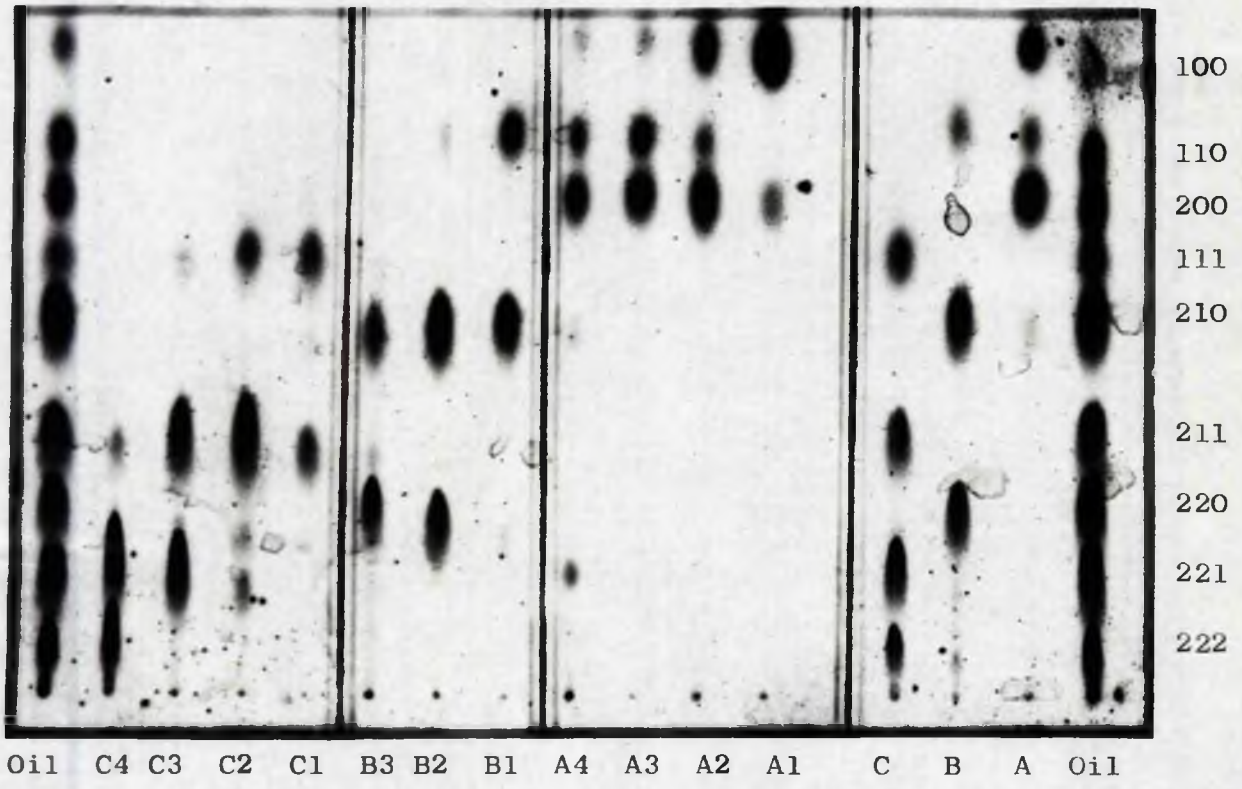


Figure 3

It is possible, from the content of saturated, oleic, and linoleic esters, to calculate mixtures containing only two or three glycerides but the fractions containing four to five glycerides are usually very small and assumptions can be made about their composition without introducing any serious errors.

The component glycerides of J. curcas seed oil are calculated below and the glyceride composition of the other seed oils are obtained in the same way.

Fraction A1

Esters (% mol.) sat. 2.3%, oleate 1.2% and linoleate 0.2%. Glycerides present (from T.L.C.) 100, 110 and 200

If the content of these three glycerides be 3A (100), 3B (110) and 3C (200) then we can write three equations:

$$2A + B + 2C = 2.3$$

$$A + 2B = 1.2$$

$$C = 0.2$$

whence 3A = 2.5, 3B = 0.6, and 3C = 0.6

Fraction A2

Esters (% mol.) sat. 2.5%, oleate 0.8%, and linoleate 0.9%. Glycerides present (from T.L.C.) 110 (3A), 200 (3B), and 100 (3C). Solving three equations similar to those described under Fraction A1:

whence 3A = 0.9, 3B = 2.7 and 3C = 0.6.

Fraction A3

Esters (% mol.) sat. 4.0%, oleate 3.1% and linoleate 1.7%.

Glycerides present (from T.L.C.) 200 (3A), 110 (3B) and 210 (3C):

whence 3A = 3.3, 3B = 3.6 and 3C = 1.9

Fraction A4

Esters (% mol.) sat. 1.2%, oleate 1.1% and linoleate 1.1%. Glycerides present (from T.L.C.) 200, 110 and 220. The chromatogram shows 210 as the major component and 200, 110 and 220 as minor components. Calculationsgive a value of 0.3% for 200 and it is assumed that 110 and 220 have the same value thus:-

200	0.3% mol.
110	0.3% "
220	0.3% "
210	2.5% "

Fraction B1

Esters (% mol.) sat. 5.5%, oleate 8.0% and linoleate 3.7%. Glycerides present (from T.L.C.) 210 and 110.

This fraction contains only the two components 110 and 210. The content of saturated esters should therefore be 5.7% and the values of oleate and linoleate are adjusted to 7.9 and 3.6%. These values indicate 6.4% of 110 and 10.8% of 210.

Fraction B2

Esters (% mol.) sat. 5.2%, oleate 3.0% and linoleate 7.6%. Glycerides present (from T.L.C.) 210 and 110. Since this fraction contains 210 and 110 the component glycerides can be calculated from its component esters thus:-

210 = 8.9% and 110 = 6.9%

Fraction B3

Esters (% mol.) sat. 1.2%, oleate 0.6% and linoleate 2.4%. Glycerides present (from T.L.C.) 220, 221 and 222.

The composition of this small fraction does not agree with the three glycerides shown to be present in the T.L.C. Based on the appearance of the chromatogram it is assumed that 220 represents half of this fraction:

whence 220 = 2.1%, 221 = 1.2% and 222 = 0.9%

Fraction C1

Esters (% mol.) sat. 0.3%, oleate 11.5% and linoleate 2.0%. Glycerides present (from T.L.C.) 111 (3A), 211 (3B) and 210 (3C):

whence 3A = 7.8, 3B = 5.1, and 3C = 0.9

Fractions C2-C4

In these fractions the small amount of saturated esters is neglected. C2 and C3 are mixtures of 211 and 221 and C4 of 221 and 222:

whence	C2	211	=	8.7% and 221	=	2.1%
	C3	211	=	1.3% and 221	=	3.8%
	C4	221	=	10.2% and 222	=	2.3%

TABLE 3 JATROPHIA CURCAS SEED OIL

Source of Seeds Director of Forest Research, Ibadan, Nigeria.

Extraction of Oil and Purification of Triglycerides

Oil extracted: 49% (based on kernels), 30% (based on seeds)

Recovered after neutralisation (99%); contained triglycerides (99%) and only traces of di- and monoglycerides.

Low Temperature crystallisation

Fraction				Wt(g.)	Wt(%)
A	Insoluble	at	-10°	0.53	20.4
B	Insoluble	at	-70°	0.97	37.1
C	Mother Liquor	at		1.11	42.5
	Loss			0.02	

Component esters (% wt.)

	14:0	16:0	18:0	16:1	18:1	18:2*
OIL	Tr	15.4	6.5	1.3	40.2	36.6
A	1.8	32.6	17.7	1.4	28.3	18.2
B	0.1	24.1	8.8	2.0	30.1	34.9
C		0.5	-	0.9	54.0	44.6

* These figures refer to the number of carbon atoms and double bonds per molecule; thus 18:2 represents octadecadienoic acid.

TABLE 4

Column Chromatography

FN.	Eluting solvent*		Weight		Component esters (% wt.)					
		ml.	mg.	%	14:0	16:0	18:0	16:1	18:1	18:2
A_1	PB50	100	22	3.3	0.4	39.8	20.3	2.3	32.0	5.2
A_2	PB70	100	24	4.2	-	42.0	18.9	0.8	17.9	20.4
A_3	B	200	51	9.0	-	27.9	17.0	1.5	34.6	19.0
A_4	E	250	20	3.4	0.2	21.0	15.3	1.2	31.0	31.3
B_1	B	200	49	17.2	0.2	22.4	8.4	2.2	46.2	20.6
B_2	BE_5	200	44	15.7	0.3	24.1	8.0	1.8	18.4	47.4
B_3	E	200	12	4.2	-	20.0	7.0	1.7	14.6	56.7
C_1	BE_2	300	35	13.9	0.2	1.6	-	1.7	83.2	13.3
C_2	BE_2	100	27	10.8	0.1	1.0	-	2.0	59.3	37.6
C_3	BE_5	200	13	5.1	-	2.3	-	2.0	41.0	54.7
C_4	E	200	32	12.7	0.1	0.9	-	1.4	26.4	71.2

Iodine value of triglycerides = 97.8 (calc. from above results 99.4)

* P = petrol (b.p. 40-60°) B = benzene, E = ether, PB70 signifies a mixture of petrol (30 vols.) and benzene (70 vols.), other symbols of this type are interpreted in this way.

TABLE 5

	Increments (% wt.)						Component esters (% mol. corrected)				
Fn	% wt.	16:0	18:0	16:1	18:1	18:2	% mol.	16:0	18:0	16:1 + 18:1	18:2
A1	3.8	1.5	0.8	0.1	1.2	0.2	3.7	1.6	0.7	1.2	0.2
A2	4.2	1.3	0.8	-	0.7	0.9	4.2	1.3	0.7	0.8	0.9
A3	9.0	2.5	1.5	0.2	3.1	1.7	8.8	2.6	1.4	3.1	1.7
A4	3.4	0.7	0.5	-	1.1	1.1	3.4	0.7	0.5	1.1	1.1
B1	17.2	3.9	1.4	0.4	8.0	3.5	17.2	4.1	1.4	8.0	3.7
B2	15.7	3.3	1.3	0.3	2.9	7.4	15.8	4.0	1.2	3.0	7.6
B3	4.2	0.8	0.3	0.1	0.6	2.4	4.2	0.9	0.3	0.6	2.4
C1	13.9	0.2	-	0.2	11.6	1.9	13.3	0.3	-	11.5	2.0
C2	10.8	0.1	-	0.2	6.4	4.1	10.8	0.1	-	6.4	4.3
C3	5.1	0.1	-	0.1	2.1	2.8	5.1	0.1	-	2.1	2.9
C4	12.7	0.1	-	0.2	3.4	9.0	13.0	0.1	-	3.4	9.5
Total		15.5	6.0	1.8	41.1	35.0		16.3	6.2	41.2	36.3

Adjustment of linoleic content (L) 36.6/35.0 = 1.05

Other acids (K) 63.4/65.0 = 0.98

TABLE 6

Component Glycerides (% mol.)

Fn	% mol.	0	1	2	100	200	110	210	220	111	211	221	222*
A1	3.7	2.3	1.2	0.2	2.5	0.6	0.6						
A2	4.2	2.5	0.8	0.9	0.6	2.7	0.9						
A3	8.8	4.0	3.1	1.7		3.3	3.6	1.9					
A4	3.4	1.2	1.1	1.1		0.3	0.3	2.5	0.3				
B1	17.2	5.5	8.0	3.7			6.4	10.8					
B2	15.8	5.2	3.0	7.6				8.9	6.9				
B3	4.2	1.2	0.6	2.4					2.1			1.2	0.9
C1	13.8	0.3	11.5	2.0					0.9	7.3	5.1		
C2	10.8	0.1	6.4	4.3							3.7	2.1	
C3	5.1	0.1	2.1	2.9							1.3	3.8	
C4	13.0	0.1	3.4	9.5								10.2	2.8
				Total	3.1	6.9	11.8	24.1	10.2	7.8	15.1	17.3	3.7

*These figures indicate the number of double bonds in the three acyl chains.

Each glyceride category includes all positional isomers.

TABLE 7

JATROPHA MULTIFIDA SEED OIL

Source of Seeds Ghana

Extraction of Oil and Purification of Triglycerides

Oil extracted: 52% (based on kernels), 40% (based on seeds)

Recovered after neutralisation (99%); contained triglycerides (80%), diglycerides (19%) and monoglycerides (1%)

Low Temperature Crystallisation

Fraction		Wt.(g.)	Wt.(%)
A*	Insoluble at -10°	0.18	12.9
B	Insoluble at -70°	0.72	52.7
C	Mother Liquor	0.47	34.4
	Loss	0.01	

Component esters (% wt.)

	16:0	18:0	16:1	18:1	18:2
Oil	16.9	7.7	1.5	23.5	50.4
A*	38.4	21.5	1.8	12.9	25.4
B	22.5	8.7	1.1	20.5	47.2
C	1.6	-	1.5	32.2	64.7

*Fraction A was not further separated by column chromatography.

TABLE 8

Column Chromatography

Fn	Eluting	solvent* ml.	Weight mg.	Weight %	Component esters (% wt.) 16:0	18:0	16:1	18:1	18:2
B1	B	200	48	18.7	26.4	11.4	2.2	37.1	22.9
B2	B	100	18	6.9	17.7	10.3	2.0	35.1	34.9
B3	BE5	200	59	23.2	24.8	9.3	1.6	4.8	59.5
B4	E	250	10	3.9	8.0	3.3	1.2	13.2	74.3
C1	BE2	200	21	6.7	7.3	-	2.5	64.4	25.8
C2	BE5	300	25	8.0	3.2	-	2.6	51.2	43.0
C3	BE10	200	13	4.1	1.0	-	1.8	35.3	61.9
C4	E	300	48	15.6	1.6	-	1.4	15.0	82.0

Iodine value of triglycerides = 107.3 (calc. from above results 109.3).

*See footnote to Table 4

TABLE 9

		Increments (% wt.)					Component esters (% mol. corrected)					
Fn	% wt.	16:0	18:0	16:1	18:1	18:2	% mol.	16:0	18:0	16:1	18:1	18:2
A	12.9	4.9	2.8	0.2	1.7	3.3	12.5	4.9	2.4	0.3	1.5	3.4
B1	18.7	5.0	2.1	0.4	6.9	4.3	18.1	5.0	1.9	0.4	6.3	4.5
B2	6.9	1.2	0.7	0.2	2.4	2.4	6.8	1.2	0.7	0.1	2.3	2.5
B3	23.2	5.8	2.2	0.3	1.1	13.8	23.6	5.8	1.9	0.4	1.0	14.5
B4	3.9	0.3	0.1	0.1	0.5	2.9	4.0	0.3	0.1	0.1	0.5	3.0
C1	6.7	0.5	-	0.2	4.3	1.7	6.5	0.5	-	0.2	4.0	1.8
C2	8.0	0.3	-	0.2	4.1	3.4	8.0	0.3	-	0.2	3.8	3.7
C3	4.1	Tr	-	0.1	1.4	2.6	4.2	Tr	-	0.1	1.4	2.7
C4	15.6	0.3	-	0.2	2.3	12.8	16.3	0.2	-	0.3	2.2	13.6
Total		18.3	7.9	1.9	24.7	47.2		18.2	7.0	2.1	23.0	49.7

Adjustment of linoleic content (K) 50.4/47.2 ≡ 1.07

Other acids (L) 49.6/52.8 ≡ 0.94

JATROPHA MULTIFIDA SEED OIL

SEPARATION OF GLYCERIDES BY LOW TEMPERATURE CRYSTALLISATION (Acetone-Methanolic $AgNO_3$) AND BY COLUMN CHROMATOGRAPHY ON SILICA-$AgNO_3$.

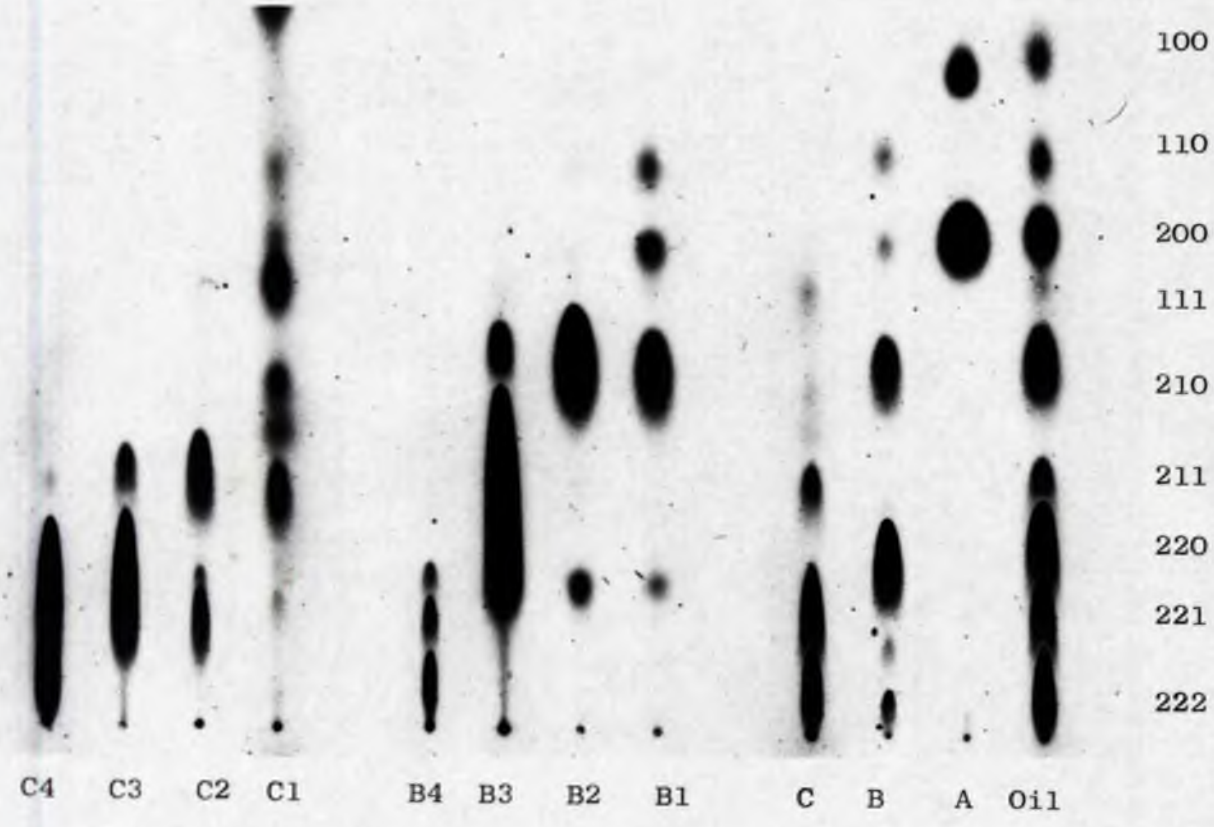


Figure 4

TABLE 10

Component Glycerides (% mol.)

Fn	% mol.	0	1	2	100	200	110	210	220	111	211	221	222*
A	12.5	7.3	1.8	3.4	4.2	8.3							
B1	18.1	6.9	6.7	4.5		2.5	4.5	11.1					
B2	6.8	1.9	2.4	2.5				6.0	0.8				
B3	23.6	7.7	1.4	14.5				4.2	19.4				
B4	4.0	0.4	0.6	3.0					1.0			1.8	1.2
C1	6.5	0.5	4.2	1.8				1.5		1.1	3.9		
C2	8.0	0.3	4.0	3.7					0.9		4.9	2.2	
C3	4.2	Tr	1.5	2.7					Tr		0.3	3.9	
C4	16.3	0.2	2.5	13.6					0.8			7.5	3.2
				Total	4.2	10.8	4.5	22.8	22.7	1.1	9.1	15.4	9.4

*See footnote to Table 6.

TABLE 11

JATROPHA GOSSYPIFOLIA SEED OIL

Source of Seeds — Ghana

Extraction of Oil and Purification of Triglycerides

Oil extracted: 27% (based on kernels).

Recovered after neutralisation (95%); contained triglycerides (92%), diglycerides (6%) and monoglycerides (2%).

Low Temperature Crystallisation

Fraction		Wt.(g.)	Wt. (%)
A*	Insoluble at -10°	0.04	2.8
B	Insoluble at -70°	0.63	45.9
C	Mother Liquor	0.70	51.3
	Loss	0.01	

Component esters (% wt.)

	14:0	16:0	18:0	16:1	18:1	18:2
Oil	0.6	7.0	5.2	0.4	16.2	70.6
A*	0.4	23.5	36.3	0.7	9.7	29.4
B	0.3	13.4	9.8	0.6	12.3	63.6
C	0.1	0.4	-	0.3	19.8	79.4

*See footnote to Table 7.

TABLE 12

Column Chromatography

Fn	Eluting solvent[+]	Weight			Component esters (% wt.)				
	ml.	mg.	%	14:0	16:0	18:0	16:1	18:1	18:2
B1	PB30	100	4	1.5	3.8	42.5	10.2	3.1	24.2	16.2
B2	B	100	6	2.2	1.4	24.2	11.0	0.3	41.8	21.3
B3	B	100	14	5.3	0.5	19.4	10.1	0.4	35.4	34.2
B4	BE5	200	66	23.8	0.4	18.3	11.8	0.2	6.9	62.4
B5	BE5	200	13	4.5	0.3	10.9	4.0	0.1	17.8	66.9
B6	E	200	24	8.6	0.2	1.5	0.4	0.5	2.2	95.2
C1	BE2	200	10	5.2	1.0	7.3	1.5	2.5	57.9	29.3
C2	BE5	200	13	6.5	-	2.8	0.9	0.8	44.8	50.7
C3	BE10	200	52	26.6	-	0.4	-	0.6	17.5	81.5
C4	E	300	25	13.0	-	0.8	-	0.4	2.7	96.1

Iodine value of triglycerides = 133.8 (calc. from above results 137.1)

[+]See footnote to Table 4.

TABLE 13

	Increments(% wt.)							Component esters (% mol. corrected)						
Fn	%wt.	14:0	16:0	18:0	16:1	18:1	18:2	% mol.	14:0	16:0	18:0	16:1	18:1	18:2
A	2,8	-	0.7	1.0	-	0.3	0.8	2.7	-	0.7	0.9	-	0.3	0.8
B 1	1.5	0.1	0.6	0.1	0.1	0.4	0.2	1.4	0.1	0.6	0.1	0.1	0.3	0.2
B2	2.2	-	0.5	0.3	-	0.9	0.5	2.2	-	0.6	0.2	-	0.9	0.5
B3	5.3	-	1.1	0.5	-	1.9	1.8	5.0	-	1.0	0.5	-	1.7	1.8
B4	23.8	0.1	4.4	2.8	-	1.6	14.9	23.7	0.1	4.4	2.5	-	1.6	15.1
B5	4.5	-	0.5	0.2	-	0.8	3.0	4.4	-	0.5	0.2	-	0.7	3.0
B6	8.6	-	0.1	-	0.1	0.2	8.2	3.9	-	0.2	-	-	0.2	3.5
C1	5.2	0.1	0.4	0.1	0.1	3.0	1.5	5.0	0.1	0.4	0.1	0.1	2.8	1.5
C2	6.5	-	0.2	-	0.1	2.9	3.3	6.4	-	0.2	-	0.1	2.7	3.4
C3	26.6	-	0.1	-	0.1	4.7	21.7	27.0	-	0.1	-	0.2	4.3	22.4
C4	13.0	-	0.1	-	-	0.4	12.5	13.3	-	0.1	-	-	0.4	12.8
Total		0.3	8.7	5.0	0.5	17.1	68.4		0.3	8.8	4.5	0.5	15.9	70.0

Adjustment of linoleic content (K) 70.6/68.4 = 1.03

Other acids (L) 29.4/31.6 = 0.93

JATROPHA GOSSYPIFOLIA SEED OIL

SEPARATION OF GLYCERIDES BY LOW TEMPERATURE CRYSTALLISATION (Acetone-Methanolic $AgNO_3$) AND BY COLUMN CHROMATOGRAPHY ON Silica-$AgNO_3$.

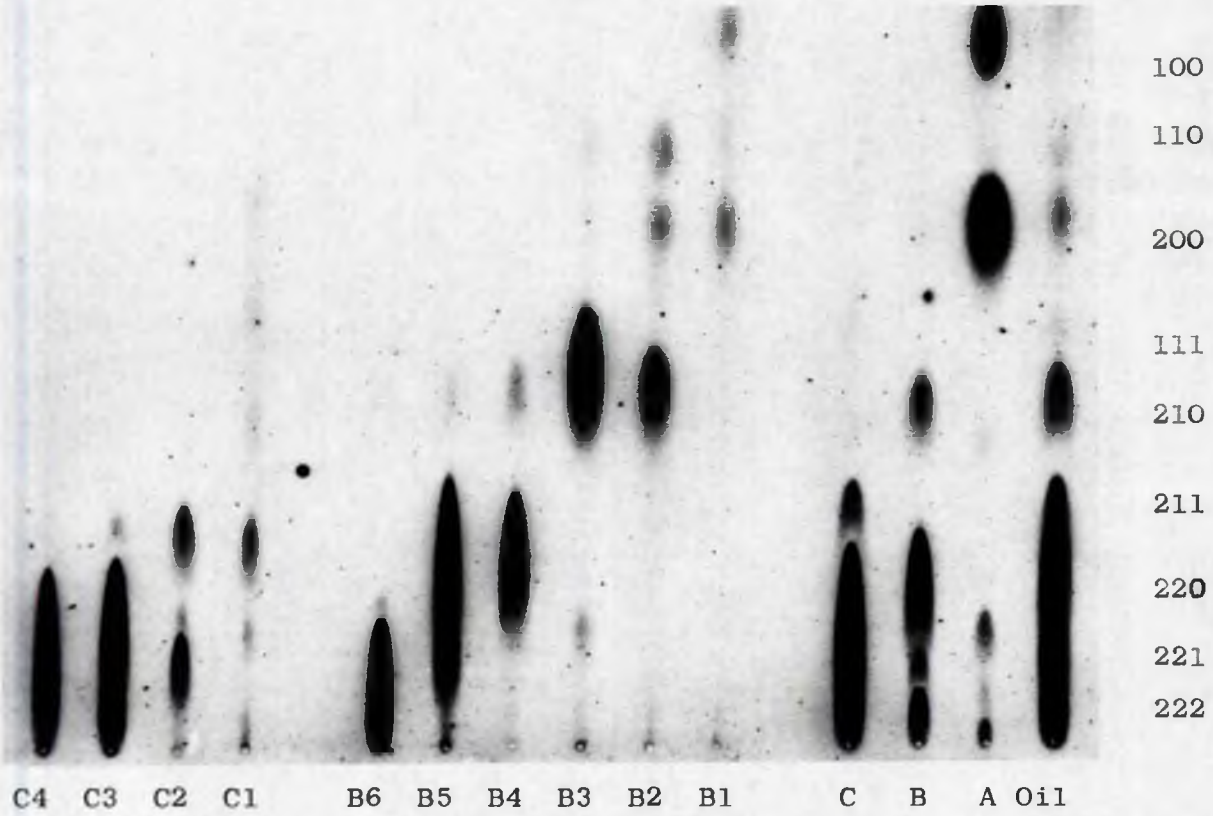


Figure 5

TABLE 14

Component Glycerides (% mol.)

Fn	% mol.	0	1	2	100	200	110	210	220	111	211	221	222*
A	2.7	1.6	0.3	0.8	0.3	1.3			0.6				
B1	1.4	0.8	0.4	0.2	0.3	0.7	0.4						
B2	2.2	0.8	0.9	0.5		0.3	0.7	1.2					
B3	5.0	1.5	1.7	1.8				4.9	0.1				
B4	23.7	7.0	1.6	15.1				0.6	20.4		1.5	1.2	
B5	4.4	0.7	0.7	3.0					2.1			2.1	0.2
B6	8.9	0.2	0.2	8.5					0.6			0.6	7.7
C1	5.0	0.6	2.9	1.5				0.9	0.9	1.1	2.1		
C2	6.4	0.2	2.8	3.4					0.6		2.8	3.0	
C3	27.0	0.1	4.5	22.4					0.3			13.4	13.3
C4	13.3	0.1	0.4	12.8					0.3			1.2	11.8
		Total			1.1	2.3	1.1	7.6	25.9	1.1	6.4	21.5	33.0

*See footnote to Table 6

TABLE 15

SUNFLOWER SEED OIL (Nigerian)

Source of Seeds — Tropical Products Institute.

Extraction of Oil and Purification of Triglycerides

Oil extracted 32% (based on kernels).

Recovered after neutralisation (99%); contained triglycerides (99%) and only traces of di- and monoglycerides.

Low Temperature Crystallisation

Fraction				Wt. (g.)	Wt. (%)
A*	Insoluble	at	-10°	0.04	1.9
B	Insoluble	at	-70°	0.67	34.9
C	Mother Liquor			1.21	63.2
	Loss			0.02	

Component esters (% wt.)

	14:0	16:0	18:0	16:1	18:1	18:2
Oil	0.2	6.6	2.6	0.4	30.3	59.7
A*	-	31.8	25.3	Tr	14.6	28.3
B	-	15.7	3.8	0.4	23.2	51.9
C	-	0.9	-	0.2	37.3	61.6

*See footnote to Table 7.

TABLE 16

Column Chromatography Fn	Eluting solvent*	ml.	Weight mg.	%	Component esters (% wt.) 14:0	16:0	18:0	16:1	18:1	18:2
B1	BP30	200	14	3.6	-	22.9	10.6	0.9	48.1	17.5
B2	B	200	26	6.9	-	19.3	10.7	0.9	34.2	34.4
B3	BE5	100	23	6.1	0.1	13.3	9.4	0.6	22.8	48.3
B4	BE5	100	31	7.9	-	16.9	9.4	0.8	16.8	56.1
B5	BE5	200	15	4.0	0.1	13.8	7.8	3.4	15.9	62.0
B6	E	200	25	6.4	-	9.4	4.6	1.0	11.2	73.8
C1	BE2	200	31	12.8	-	3.1	-	0.3	66.5	30.1
C2	BE2	200	17	7.1	-	1.9	-	0.7	50.8	46.6
C3	BE5	400	31	12.8	-	1.1	-	0.5	42.3	56.1
C4	BE5	200	15	6.0	-	0.9	-	0.3	35.2	63.5
C5	BE3	300	21	8.4	-	1.2	-	0.4	28.8	69.6
C6	E	300	39	16.1	-	0.4	-	0.2	16.3	83.1

Iodine value of triglycerides = 124.6 (calc. from above results 129.2).

*See footnote to Table 4..

TABLE 17

	Increments (% wt.)						Component esters (% mol. corrected)					
Fn	% wt.	16:0	18:0	16:1	18:1	18:2	% mol.	16:0	18:0	16:1	18:1	18:2
A	1.9	0.6	0.5	-	0.3	0.5	1.8	0.6	0.4	-	0.2	0.6
B1	3.6	0.8	0.4	-	1.8	0.6	3.4	0.8	0.3	-	1.6	0.7
B2	6.9	1.4	0.7	0.1	2.3	2.4	6.7	1.3	0.7	0.1	2.1	2.5
B3	6.1	1.2	0.6	-	1.4	2.9	6.1	1.1	0.5	-	1.3	3.2
B4	7.9	1.3	0.7	0.1	1.3	4.5	8.0	1.3	0.7	0.1	1.2	4.7
B5	4.0	0.6	0.3	-	0.6	2.5	4.1	0.5	0.3	-	0.6	2.7
B6	6.4	0.6	0.3	0.1	0.7	4.7	6.7	0.6	0.3	0.1	0.6	5.1
C1	12.8	0.4	-	-	8.5	3.9	12.1	0.4	-	-	7.6	4.1
C2	7.1	0.1	-	0.1	3.6	3.3	7.0	0.1	-	0.1	3.2	3.6
C3	12.8	0.1	-	0.1	5.4	7.2	12.7	0.1	-	0.1	4.8	7.7
C4	6.0	0.1	-	-	2.1	3.8	6.0	0.1	-	-	1.9	4.0
C5	8.4	0.1	-	-	2.4	5.9	8.6	0.1	-	-	2.2	6.3
C6	16.1	0.1	-	-	2.6	13.4	16.8	0.1	-	-	2.4	14.3
Total		7.4	3.5	0.5	33.0	55.6		7.1	3.2	0.5	29.7	59.5

Adjustment of linoleic content (K) $59.7/55.6 \equiv 1.07$

Other acids (L) $40.3/44.4 \equiv 0.91$

SUNFLOWER SEED OIL (NIGERIAN)

SEPARATION OF GLYCERIDES BY LOW TEMPERATURE CRYSTALLISATION (ACETONE-METHANOLIC $AgNO_3$) AND BY COLUMN CHROMATOGRAPHY ON SILICA-$AgNO_3$.

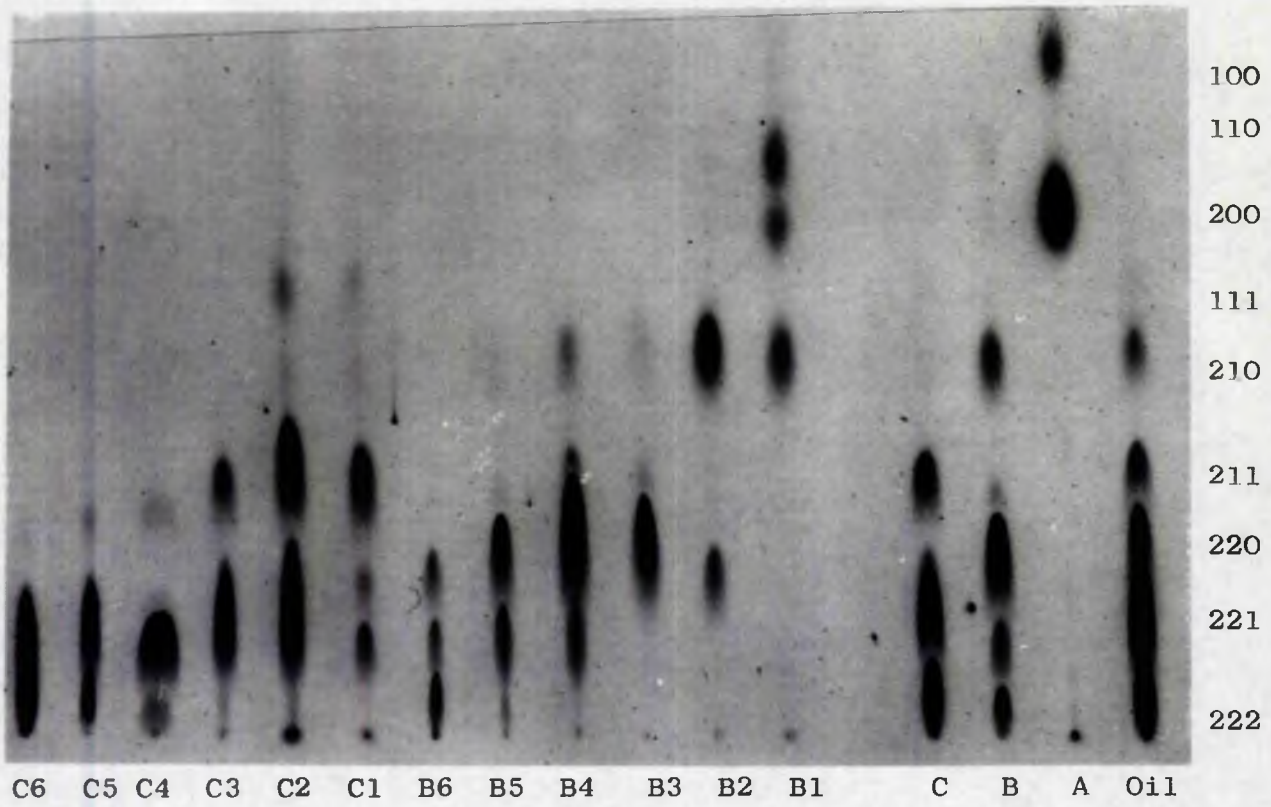


Figure 6

TABLE 18

Component Glycerides (% mol.)

Fn	% mol.	0	1	2	100	200	110	210	220	111	211	221	222*
A	1.8	1.0	0.2	0.6	0.4	1.1					0.1	0.1	0.1
B1	3.4	1.1	1.6	0.7			1.3	2.1					
B2	6.7	2.0	2.2	2.5				5.1	0.9		0.7		
B3	6.1	1.6	1.3	3.2				1.5	3.4		1.2		
B4	8.0	2.0	1.3	4.7				1.7	4.2			2.1	
B5	4.1	0.3	0.6	2.7					2.4			1.7	
B6	6.7	0.9	0.7	5.1					2.7			2.1	1.9
C1	12.1	0.4	7.6	4.1				1.1		Tr	10.7	0.3	
C2	7.0	0.1	3.3	3.6					0.3	Tr	3.3	3.4	
C3	12.7	0.1	4.9	7.7					0.3		2.4	10.0	
C4	6.0	0.1	1.9	4.0					0.3			5.6	0.1
C5	8.6	0.1	2.2	6.3					0.3			6.6	1.7
C6	16.8	0.1	2.4	14.3					0.3			7.2	9.3
				Total	0.4	1.1	1.3	11.5	15.1	Tr	18.4	39.1	13.1

*See footnote to Table 6.

TABLE 19

SUNFLOWER SEED OIL (Nigerian)

Source of Seeds — Tropical Products Institute

Extraction of Oil and Purification of Triglycerides

Oil extracted 33% (based on kernels)

Recovered after neutralisation (99%); contained triglycerides (99%) and only traces of di- and monoglycerides.

Low Temperature Crystallisation

Fraction		Wt. (g.)	Wt. (%)
A*	Insoluble at -10°	0.02	1.3
B	Insoluble at -70°	0.32	23.8
C	Mother Liquor	0.99	74.9
	Loss	0.01	

Component esters (% wt.)

Oil	14:0	16:0	16:1	18:1	18:2
	0.1	4.7	1.8	0.5	50.7	42.2
A*	-	12.6	11.8	0.5	41.3	33.3
B	0.1	16.8	9.9	0.4	37.4	35.8
C		0.3	-	0.1	56.9	42.7

*See footnote to Table 7.

TABLE 20

Column Chromatography

Fn	Eluting solvent*		Weight		Component esters (% wt.)				
		ml.	mg.	%	16:0	18:0	16:1	18:1	18:2
B1	BP30	200	44	6.6	21.1	10.6	0.4	55.1	12.8
B2	B	200	34	5.5	18.4	9.5	0.6	41.2	30.3
B3	BE5	200	37	6.2	17.5	9.6	0.3	30.4	42.2
B4	BE5	200	14	2.4	16.1	8.8	0.7	20.9	53.5
B5	BE10	200	9	1.4	13.7	8.1	0.8	19.5	57.9
B6	E	300	11	1.7	12.0	6.0	1.4	15.0	65.6
C1	BE2	200	31	20.0	1.1	-	0.3	85.5	13.1
C2	BE2	200	15	9.8	0.3	-	0.1	73.1	26.5
C3	BE5	200	39	24.8	0.6	-	0.3	53.2	45.9
C4	BE10	400	20	12.4	0.7	-	0.3	32.8	66.2
C5	E	200	12	7.9	1.1	-	0.6	25.1	73.2

Iodine value of triglycerides = 111.9 (calc. from above results 116.3)

*See footnote to Table 4.

TABLE 21

	Increments (% wt)						Component esters (% mol. corrected)					
Fn	% wt.	16:0	18:0	16:1	18:1	18:2	% mol.	16:0	18:0	16:1	18:1	18:2
A	1.3	0.2	0.2	-	0.5	0.4	1.3	0.2	0.1	-	0.5	0.5
B1	6.6	1.4	0.7	Tr	3.6	0.9	6.5	1.4	0.7	-	3.5	0.9
B2	5.5	1.0	0.5	Tr	3.2	1.7	5.5	1.0	0.5	-	2.2	1.8
B3	6.2	1.1	0.5	Tr	1.9	2.6	6.2	1.1	0.5	-	1.3	2.8
B4	2.4	0.4	0.2	Tr	0.5	1.3	2.4	0.4	0.2	-	0.5	1.3
B5	1.4	0.2	0.1	Tr	0.3	0.8	1.5	0.2	0.1	-	0.3	0.9
B6	1.7	0.2	0.1	Tr	0.2	0.2	1.8	0.2	0.1	-	0.3	1.2
C1	20.0	0.2	-	0.1	17.1	2.6	19.2	0.2	-	0.1	16.1	2.8
C2	9.8	-	-	-	7.2	2.6	9.7	-	-	-	6.8	2.9
C3	24.8	0.1	-	0.1	13.2	11.4	24.9	0.1	-	0.1	12.5	12.2
C4	12.4	0.1	-	-	4.1	8.2	12.8	0.1	-	-	3.9	8.8
C5	7.9	0.1	-	-	2.0	5.8	8.2	0.1	-	-	1.9	6.2
	Total	5.0	2.4	0.2	52.9	39.5		5.0	2.2	0.2	50.3	42.3

Adjustment of linoleic content (K) 42.2/39.5 = 1.07

Other acids (L) 57.8/60.5 = 0.95

SUNFLOWER SEED OIL (NIGERIAN)
SEPARATION OF GLYCERIDES BY LOW TEMPERATURE CRYSTALLISATION (Acetone-Methanolic $AgNO_3$) AND BY COLUMN CHROMATOGRAPHY ON Silica-$AgNO_3$.

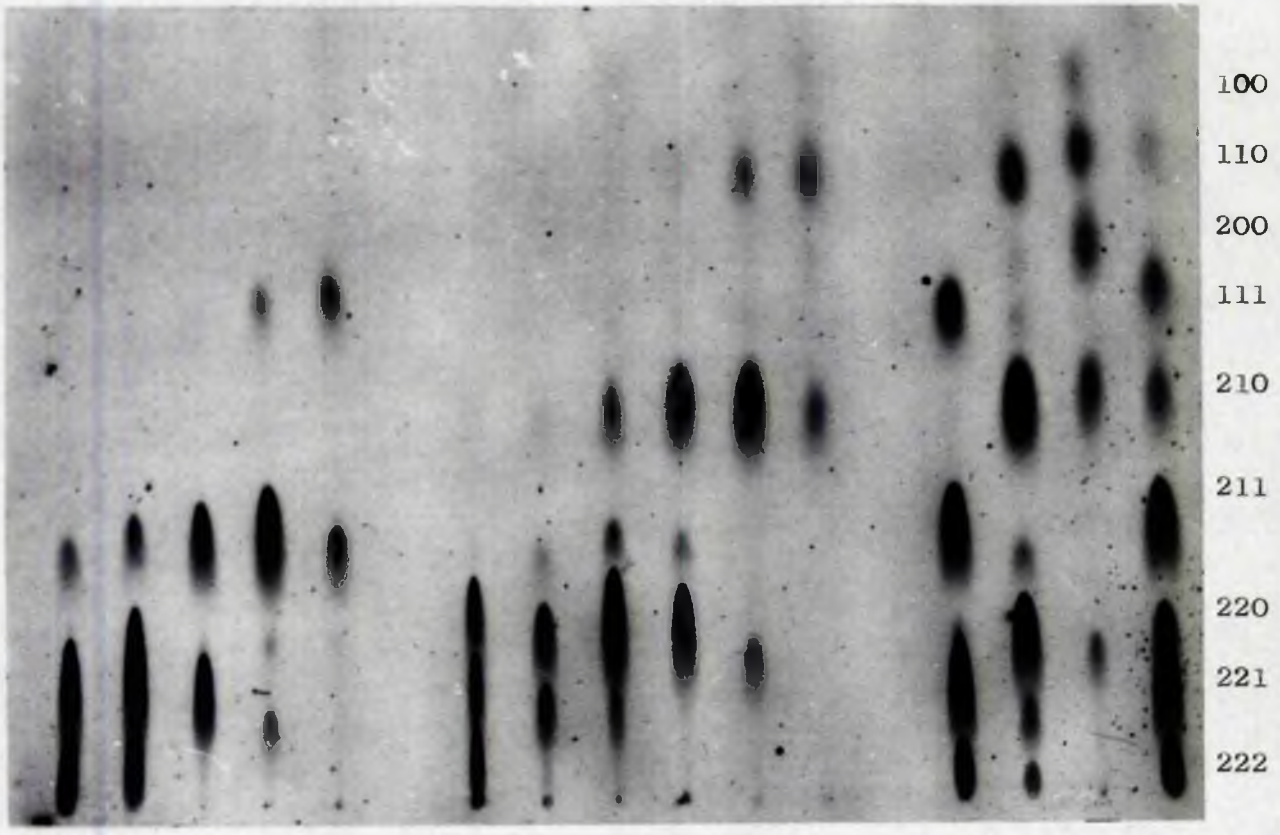


Figure 7

TABLE 22

Component Glycerides (% mol.)

Fn	% mol.	O	1	2	100	200	110	210	220	111	211	221	222*
A	1.3	0.3	0.5	0.5	0.3	0.5		0.5					
B1	6.5	2.1	3.5	0.9			3.3	2.7					
B2	5.5	1.5	2.2	1.8			0.6	4.4	0.5				
B3	6.2	1.6	1.3	2.3				3.1	3.1				
B4	2.4	0.6	0.5	1.3				0.5	1.3		0.3	0.3	
B5	1.5	0.3	0.3	0.9					0.8			0.7	
B6	1.8	0.3	0.3	1.2					0.6			0.6	0.6
C1	19.2	0.2	16.2	2.8					0.6	11.4	7.2		
C2	9.7	-	6.8	2.9						1.0	8.7		
C3	24.9	0.1	2.6	12.2							13.1	11.8	
C4	12.8	0.1	3.9	8.3								11.3	1.0
C5	8.2	0.1	1.9	6.2								5.7	2.5
				Total	0.3	0.5	4.4	11.2	6.9	12.4	29.3	30.9	4.1

*See footnote to Table 6.

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PART II

Determination of Component Glycerides by Thin Layer Chromatograohy on Silica Impregnated with Silver Nitrate.

PART II

Determination of Component Glycerides by Thin Layer Chromatography on Silica Impregnated with Silver Nitrate.

1. DISCUSSION

(i) Methods

The triglyceride composition of natural fats has been studied by fractional crystallisation[1-6] countercurrent distribution;[7-12] thermal gradient crystallisation;[13,14] liquid liquid partition chromatograohy[15,18] and column,[19] paper[20] and thin layer chromatography.[21-23] More detailed information has been calculated from enzymic hydrolysis methods,[24-27] either applied directly to the oil or after fractionation. The separation of triglycerides according to mol. wt. by means of gas liquid chromatography[28,29] has proved useful in combination with other separation techniques.[30]

The fractionation procedures have provided some insight into the composition of triglycerides but have failed to distinguish between isomeric and unsaturated glycerides. However, Barrett et al.[31] separated some glycerides according to unsaturation by thin layer argentation procedure.

The ability of olefins to form co-ordination complexes with certain metal ions has long been recognised and the compound formed between the silver ions and variety of unsaturated hydrocarbons have been studied extensively by Lucas and his co-workers.[32-34] Dutton et al.[35] separated methyl

cis-octadecenoate (oleate) and methyl trans-octadecenoate (elaidate) by means of counter-current distribution between light petroleum ether and methanolic silver nitrate, de Vries[36,37] was able to separate cis and trans isomers on a column of silica impregnated with silver nitrate. Barrettet al.[31] reported the resolution of 2-linolein-1,3-distearin from 1-linolein-2,3-distearin and separated cottonseed oil into five spots.

We have extended and improved the method of Barrett et al.[31] The crushed seeds (safflower, tobacco, Argemone mexicana, maize, cotton, groundnut, Macadamia ternifolia, Gmelina asiatica and Madhuca latifolia) were thoroughly extracted with boiling petrol ether (b.p. 40-60°) and the extracted oils neutralized by percolating a chloroform solution through a column of alumina. The triglycerides were subsequently separated from partial glycerides by eluting from a column of silica gel (Whatman chromedia SG31). The triglycerides (15-20 mg., in 10% petrol ether solution) were applied as a band to glass plates (20 x 40 cm) layered with silica gel[38] (Merck, Darmstadt, Kieselgel G.) containing 12.5% of silver nitrate and developed with benzene ether (9:1) by a continuous development technique[39] for 2½ to 3 hours. The technique of continuous development is based on the principle of allowing the solvent to pass from one end of the thin layer to the other end where it is allowed to evaporate.

The separations improved considerably when the size of the plates (20 x 40 cm.) as well as the developing time was increased.

In the initial exploratory work, a number of developing solvents were examined. Clear separations of saturated glycerides were obtained with chloroform and (0.5%) acetic acid but the unsaturated glycerides were not clearly resolved due to tailing. The mixture of carbon tetrachloride and chloroform (60:40), recommended by Barrett et al.,[31] separated glycerides having different numbers of double bonds but gave only limited separation of glycerides of equal unsaturation. There was some indication, however, of separation of glycerides of equal unsaturation which yet contain different acids (e.g. 220 from 211). Improved separation of such glycerides were achieved with benzene-ether (9:1) which, however, was less effective than carbon tetrachloride-chloroform in separating the symmetrical and unsymmetrical distearo-oleins.

Natural fats which contain palmitic acid, hexadecenoic acid, stearic acid, oleic acid and linoleic acid were separated into nine zones corresponding to trilinolein (222), dilinoleo-olein (221), saturated dilinoleins (220), linoleo-diolein (211), saturated linoleo-oleins (210), triolein (111), disaturated linoleins (200), saturated dioleins (110) and disaturated oleins (100). The glycerides with the same total unsaturation such as 220 and 211, 210 and 111, and 200 and 110 were adequately separated. In the case of Cmelina asiatica, which contained 10% of eicosenoic acid (E), the glycerides 211 and 21E, 111 and 11E and 110 and 1E0 were also resolved.

In the triglyceride pairs 220 and 211, 210 and 111 and 200 and 110, the complex formation of the silver ions with a linoleic chain is apparently stronger than with two oleic chains. In the case of Gmelina asiatica seed oil the subfraction in 221 and 22E glycerides was less distinct but it was quite apparent in the case of 210 and 2EO, 111 and 11E, and 110 and 1EO.

For quantitative analysis of triglycerides by thin layer argentation procedure, dichlorofluorescein was found to be the most suitable spray reagent for detecting zones. With dichlorofluorescein the bands containing as little as 0.1 mg. were detected and analysed (see page 116). On silver nitrate impregnated plates the separated glycerides are not satisfactorily detected by iodine vapour, rhodamine B, or Sudan Black.

The separated triglycerides were recovered from the adsorbent by repeated extraction (6 x 10 ml.) with a mixture of ether, benzene and water (5:5:1). The adsorbents containing the triglycerides were placed in centrifuge tubes, stirred with the extracting liquids, then centrifuged and decanted. This method proved very effective, especially for unsaturated glycerides which adhere very strongly to the silica-silver nitrate.

It was found to be quicker and more effective than soxhlet extraction or percolation of solvent through a column containing adsorbed glycerides.

The effectiveness of this method is apparent from Table 1, where the component esters (% mol.) of whole triglycerides are

TABLE 1

Component Esters (% mol.) of the Whole Oil and the Sum of the Separated Fractions.

	14:0	16:0	18:0	16:1	18:1	18:2	18:3*
Safflower							
triglycerides	-	6.6	4.4	0.6	12.2	77.0	0.2
plate(9 fns.)	-	7.0	2.8	1.0	12.8	76.0	0.4
Tobacco							
triglycerides	-	9.8	3.8	1.0	13.5	70.6	1.3
plate(9fns.)	-	11.3	4.6	0.6	14.2	68.5	0.8
A. mexicana							
triglycerides	-	12.3	4.2	0.3	28.1	55.1	-
plate(9 fns.)	-	12.5	4.7	1.3	27.6	53.9	-
Maize							
triglycerides	-	12.6	1.8	0.8	30.0	54.3	0.5
plate(9 fns.)	-	14.4	2.5	1.0	29.1	52.0	1.0
Cotton							
triglycerides	1.1	27.3	3.1	1.4	16.7	50.4	-
plate(9fns.)	1.6	27.3	2.8	2.0	17.0	49.3	-
plate(9fns.)	1.6	26.6	4.6	2.7	16.7	47.8	-
Groundnut							
triglycerides[1]	-	9.8	3.7	0.4	60.9	18.1	-
plate(9 fns.)	-	10.5	2.8	0.7	59.8	18.6	-
M. ternifolia							
triglycerides[2]	0.7	9.3	3.7	27.2	51.9	2.8	-
plate(7 fns.)[2]	0.7	10.0	3.2	28.3	50.9	3.2	-
M. latifolia							
triglycerides	-	23.7	24.1	0.2	37.6	14.4	-
plate(9 fns.)	-	23.1	25.8	0.6	37.2	13.3	-
G. asiatica							
triglycerides[3]	-	10.1	8.1	0.3	28.8	37.6	-
plate(12 fns.)[3]	-	10.0	7.6	0.5	29.4	32.7	-

Table 1 contd.

* These figures refer to the number of carbon atoms and double bonds per molecule; thus 18:2 represents octadecadienoic acid.

	20:0	22:0	24:0	20:1	22:1
1 also	1.4	2.7	1.4	1.3	0.3
	1.5	2.8	-	0.8	0.2
2 also	2.4	-	-	2.0	-
	2.8	-	-	0.9	-
3 also	2.8	2.4	-	9.9	0.2
	2.6	1.4	-	8.5	0.3

compared with those recovered from the plates. The moderately good agreement indicates that our recovery is quite high (87-93%). However, the linoleic acid contents are slightly lower than the original values, while the values of the other acids are high. This loss in linoleic acid is probably due to the difficulty of recovering the highly unsaturated glycerides from the silica silver nitrate adsorbent to which it adheres very strongly.

The extracts were too small to be weighed with convenience so a known amount of methyl heptadecanoate (0.2-1 mg.) was added to each extract before it was converted to methyl esters for quantitative examination by gas liquid chromatography. From the peak areas for each ester and the C17 ester the composition of each fraction and its relative amount was determined. Each extract usually contains one major triglyceride accompanied by minor amounts of one or two others and its glyceride composition can be derived from the molar proportions of the component esters.

We consider this technique of adding a known amount of a marker to an extract from a thin layer chromatogram to be more reliable than the chromotropic colour reaction of Van-Handel[40] or glycerol determination with periodate acid after saponification.[41]

The technique could be extended to other quantitative studies based on separations by thin layer chromatography.

The efficiency of thin layer argentation procedure is apparent in Table 2, which shows the concentration of each glyceride or group of glycerides in the fraction in which it

TABLE 2

Concentration (% mol.) of Glycerides in Individual Fractions

	222	221	220	221	210	111	200	110	100*
safflower	94	93	90	72	83	55	87	78	61
tobacco	80	85	93	79	76	87	67	35	-
A. mexicana	92	93	92	84	80	88	71	88	65
maize	68	82	83	92	92	66	81	41	39
cotton	74	65	92	57	95	-	87	65	89
cotton	73	69	90	-	93	-	86	45	81
groundnut	-	59	69	95	80	86	-	92	79
M. ternifolia	-	-	-	61	48	80	-	70	50
M. latifolia	-	-	-	50	75	40	-	52	94
G. asiatica	82	91	88	98	94	-	78	95	82

* These figures indicate the number of double bonds in the three acyl chains. Each glyceride category includes all positional isomers.

predominates. Most of these values exceed 80%, many exceed 90%, and lower values generally relate to minor glycerides (5% or below) which could give only high concentration in smaller fraction than we choose to select. We believe that if necessary these values could be increased and that thin layer argentation procedure provides an excellent method for the isolation and purification of glycerides.

The reproducibility of the results obtained by thin layer argentation is also apparent from the component glycerides of cottonseed oil which we determined twice. There is good agreement between both results which are given in Tables 12 and 14.

(ii) Results

Results reported in Tables 3-24, are discussed, along with results obtained by other methods on page 139 , Part III.

(iii) Comments

The thin layer argentation procedure has proved an excellent tool for the separation of glycerides according to their degree of unsaturation. The method is simple, rapid, inexpensive and versatile. It requires very little working material and it can be extended to the characterisation of modified natural oils and synthetic mixtures.

However, the present method is only suitable for oils containing a limited number of saturated and unsaturated acids. The complete analysis of oils containing a large number of saturated acids such as coconut and palm kernel, is not possible. A suitable method, for the isomeric glycerides

is also required.

2. EXPERIMENTAL

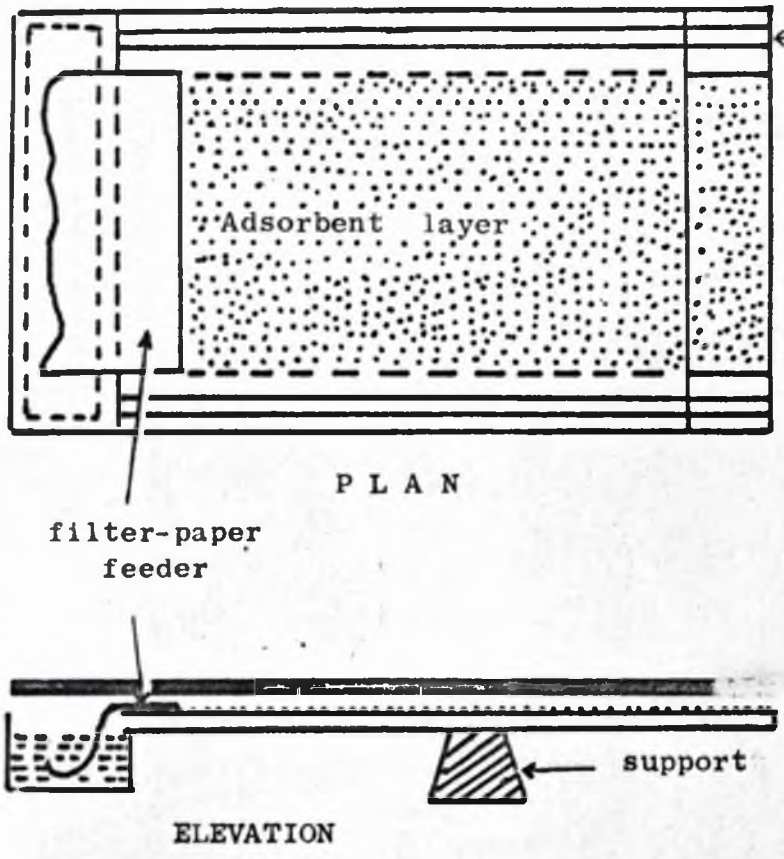
Wherever possible operations were carried out under nitrogen and all glycerides, esters and acids were stored under nitrogen at 0°. The solvents used were purified and distilled according to Vogel.[42]

Crushed seeds were thoroughly extracted with boiling petrol ether (b.p. 40-60°). The extracted oils were neutralised by percolation in chloroform through columns of alumina. The triglycerides were subsequently eluted from columns of silica (Whatman chromedia, SG31) with benzene; more polar solvents removed diglycerides and monoglycerides[43] (for details see Experimental, Part I, page 59).

Quantitative Analysis of Triglycerides by Thin Layer Argentation Method.

Glass plates (20 x 40 cm.) were layered with silica silver nitrate slurry[31,38] (see page 62), dried and activated by heating at 110° for half an hour before use.

Two strips of silica gel about 1 cm. wide were removed from both edges of the chromatoplate parallel to the direction of development. Solutions (10% in petrol ether) of triglycerides (15-20 mg.) were applied to the impregnated plates as a band along the base line 3-4 cm. from the lower horizontal edges of the plates and developed by continuous development[39] where the



Apparatus for continuous development

Figure 8

solvent passes along the layer and evaporates from the far end of the chromatogram.

Two glass spacer strips were placed along the edges from which the silica had been removed, but not allowed to come into contact with the adsorbent. An aluminum plate was placed on these strips to cover the solvent trough and all the plate except the far end (2 cm.). The glass and the aluminum plate were clamped together with (Foldback) paper clamps and a piece of filter paper (16 x 16 cm., previously extracted with ether) folded back about 2 cm. from one end had its folded end inserted between the plates to make contact with the adsorbent. The other end dipped into the solvent trough which contained benzene-ether (9:1). The plates were then supported horizontally and developed for 2½ to 3 hours.

After development the plates were dried in a current of nitrogen, and sprayed with 0.2% ethanolic solution of dichlorofluorescein.[44] The separated glycerides then appeared as bright yellow fluorescent zones when viewed under ultraviolet light.

The separated glycerides and adsorbent were scraped from the plate with a new razor blade, placed in marked centrifuge tubes, and subsequently extracted from the adsorbent with a mixture of ether, benzene and water (5:5:1, 6 x 10 ml.). The contents of the tubes, after stirring mechanically (2 min.) were centrifuged and decanted. To each extract, a known amount of

a methyl heptadecanoate solution (20 mg. in 100 ml.) was added. The amount was 5,2, or 1 ml. depending on the area of the extracted zone. The extracts, after pouring into water (40 ml.) to remove dissolved silver nitrate and methanol, were re-extracted with n-hexane (3 x10 ml.) and evaporated. Final traces of moisture were removed by adding dry acetone (5 ml.) and gently blowing the contents with a current of nitrogen at 80-90°C. All the triglycerides fractions, after transesterification with sodium methoxide, were analysed by gas liquid chromatography on a polyethyleneglycol succinate column (for details see Experimental Part 1, page 64).

3. CALCULATIONS AND RESULTS

Determination of % Wt. of the Fractions

Suppose a known amount of C_{17} ester is added in a fraction which contains "N" fatty esters then

$$\frac{\text{peak areas of N fatty esters}}{\text{peak area of } C_{17} \text{ ester}} = R$$

but

$$\frac{\text{peak areas of N fatty esters}}{\text{peak area of } C_{17} \text{ ester}} = \frac{\text{Wt. of N fatty esters}}{\text{Wt. of } C_{17} \text{ ester added}}$$

$$\therefore \quad \text{Wt. of N fatty esters} = R \times \text{Wt. of } C_{17} \text{ ester added.}$$

Calculations for Component Glycerides

The fractions separated by thin layer argentation procedure contain either two or three glycerides and their

composition can be determined by solving the simultaneous equations as described in Part I, page 35 .

Adjustment of 20:0, 22:0, 24:0 and 22:1 esters in Groundnut Oil

Due to an oversight the amount of 20:0, 22:0, 24:0 and 22:1 (all of them minor components) was determined in the total esters but not in the separated glycerides fractions. It was therefore assumed that the proportion of the saturated acids to 18:0 would be the same in each fraction as in the total oil and that the ratio of 22:1 to 20:1 would also be the same in the fractions as in the oil.

The following abbreviations are used in the Tables

1. 18:0, 18:1, 18:2 etc. etc. These figures refer to the number of carbon atoms and double bonds per molecule; thus 18:2 represents octadecadienoic acid.
2. 322, 222, 210 etc.etc. The figures indicate the number of double bonds in the three acyl chains. Each glyceride category includes all positional isomers.
3. E signifies eicosenoic and docosenoic esters.

TABLE 3

(i) SAFFLOWER SEED OIL

Source of Seeds Tropical Products Institute.

Extraction of Oil and Purification of Triglycerides

Oil extracted 30% (based on seeds)

Recovered after neutralisation (93%); contained triglycerides(92%), diglycerides (7%) and monoglycerides(1%).

Component Esters

	16:0	18:0	16:1	18:1	18:2	18:3
Oil (% mol.)	6.6	3.4	0.6	12.2	77.0	0.2

Component Esters of Fractions Obtained by T.L.C.

		Increments (% Wt.)							Increments (% mol.)					
FN	% Wt.	16:0	18:0	16:1	18:1	18:2	18:3	% mol.	16:0	18:0	16:1	18:1	18:2	18:3
1	50.2	0.2	-	0.1	0.7	49.2	-	50.0	0.2	-	0.1	0.7	49.0	-
2	18.0	0.4	-	0.1	5.5	12.0	-	17.8	0.4	-	0.1	5.4	11.9	-
3	16.7	3.2	1.6	0.3	0.8	10.6	0.2	16.3	3.5	1.6	0.3	0.8	10.5	0.2
4	4.0	0.3	0.1	0.1	1.9	1.6	-	4.0	0.3	0.1	0.1	1.9	1.6	-
5	6.6	1.2	0.7	0.1	2.3	2.2	0.1	6.7	1.3	0.7	0.1	2.2	2.3	0.1
6	1.2	0.2	-	-	0.8	0.2	-	1.2	0.2	-	-	0.8	0.2	-
7	1.5	0.5	0.2	0.1	0.1	0.5	0.1	1.6	0.6	0.2	0.1	0.1	0.5	0.1
8	1.2	0.3	0.1	0.1	0.7	-	-	1.2	0.3	0.1	0.1	0.7	-	-
9	0.6	0.2	0.1	0.1	0.2	-	-	0.6	0.2	0.1	0.1	0.2	-	-
Total		6.5	2.8	1.0	13.0	76.3	0.4		7.0	2.8	1.0	12.8	76.0	0.4

Iodine value of triglycerides 141.0 (calc. from above results 145.9)

TABLE 4

Component Glycerides (% mol.)

FN	% mol.	0	1	2	222	221	220	211	210	111	200	110	100
1	50.0	0.2	0.8	43.0	47.0	2.4	0.6						
2	17.8	0.4	5.5	11.9		16.8	1.2						
3	16.9	5.1	1.1	10.7			15.3	1.6					
4	4.0	0.4	2.0	1.6			0.9	2.8	0.3				
5	6.7	2.0	2.3	2.4			0.3	0.6	5.8				
6	1.2	0.2	0.8	0.2					0.6	0.6			
7	1.6	0.8	0.2	0.6						0.2	1.4		
8	1.2	0.4	0.8	-								1.2	
9	0.3	0.3	0.3	-								0.3	0.3
				Total	47.0	19.0	18.3	5.0	6.7	0.8	1.4	1.5	0.3

TABLE 5

TOBACCO SEED OIL

(ii)

Source of Oil Supplied as crude oil by Younghusband, Stephens and Co. Ltd.

Purification of Triglycerides

Recovered after neutralisation (96%), contained triglycerides (93%) diglycerides (6%) and mono-glycerides (1%)

Component Esters

	16:0	18:0	18:1	18:1	18:2	18:3
Oil (% mol.)	9.8	3.8	1.0	12.5	70.6	1.3

Component Esters of Fractions obtained by T.L.C.

	Increments (% Wt.)							Increments (% mol.)					
FN	% Wt.	16:0	18:0	18:1	18:1	18:2	18:3	% mol.	16:0	18:0	18:1	18:1	18:2	18:3
1	41.2	0.4	0.2	0.1	1.3	38.5	0.7	40.8	0.4	0.2	0.1	1.2	38.3	0.7
2	14.9	0.5	0.1	0.1	4.3	9.9	-	14.9	0.6	0.1	0.1	4.3	9.8	-
3	21.0	4.4	1.7	-	1.0	13.8	0.1	21.2	4.8	1.8	-	1.0	13.5	0.1
4	4.9	0.2	0.2	-	2.6	1.9	-	4.8	0.2	0.2	-	2.5	1.9	-
5	6.9	1.4	1.0	0.1	2.3	2.1	-	6.9	1.5	0.9	0.1	2.3	2.1	-
6	0.9	0.1	-	-	0.7	0.1	-	0.9	0.1	-	-	0.7	0.1	-
7	8.8	3.0	1.2	0.2	1.9	2.5	-	9.0	3.3	1.2	0.2	1.8	2.5	-
8	0.9	0.3	0.1	0.1	0.3	0.1	-	0.9	0.3	0.1	0.1	0.3	0.1	-
9	0.5	0.1	0.1	-	0.1	0.2	-	0.5	0.1	0.1	-	0.1	0.2	-
Total		10.4	4.6	0.6	14.5	69.1	0.8		11.3	4.6	0.6	14.2	68.5	0.8

Iodine value of triglycerides 138.3 (calc. from above results 138.7)

TABLE 6

Component Glycerides (% mol.)

FN	% mol.	0	1	2	3	332	222	221	220	211	210	111	200	110	100
1	40.9	0.6	1.3	38.3	0.7	2.1	33.1	3.9	1.8						
2	14.0	0.7	4.4	9.8	-			12.5	2.1	0.3					
3	21.2	3.6	1.0	13.5	0.1				19.5	1.4	0.3				
4	4.8	0.4	2.5	1.8	-				0.9	3.6	0.3				
5	3.9	2.4	2.4	2.1	-						5.4	0.6	0.9		
6	0.9	0.1	0.7	0.1	-						0.3	0.6			
7	9.0	4.5	2.0	2.5	-						1.5	1.5	6.0		
8	0.9	0.4	0.4	0.1	-								0.3	0.3	0.3
9	0.5	0.2	0.1	0.2	-						0.3		0.2		
			Total			2.1	33.1	16.4	24.3	5.3	2.1	2.7	7.4	0.3	0.3

TABLE 7

(iii) ARGEMONE MEXICANA SEED OIL

Source of Seeds Jamaica

Extraction of Oil and Purification of Triglycerides

Oil Extracted 39% (based on seeds)

Recovered after neutralisation (98%); contained triglycerides(88%), diglycerides (9%) and monoglycerides (2%).

Component Esters

	16:0	18:0	16:1	18:1	18:2
Oil (% mol.)	12.3	4.2	0.3	28.1	55.1

Component Esters of Fractions obtained by T.L.C.

	Increments (% Wt.)						Increments (% mol.)					
FN	% Wt.	16:0	18:0	16:1	18:1	18:2	% mol.	16:0	18:0	16:1	18:1	18:2
1	21.3	0.1	-	0.1	0.3	20.8	21.1	0.1	-	0.1	0.3	20.6
2	18.2	0.3	-	0.1	5.7	12.1	18.0	0.3	-	0.1	5.6	12.0
3	16.9	3.7	1.5	0.1	0.6	11.0	17.1	4.0	1.5	0.1	0.6	10.9
4	13.4	0.4	0.3	0.1	8.2	4.4	13.3	0.4	0.3	0.1	8.1	4.4
5	15.5	3.5	1.5	0.3	5.4	4.8	15.3	3.8	1.5	0.3	5.3	4.7
6	3.8	0.2	0.1	0.1	3.3	0.1	3.7	0.2	0.1	0.1	3.2	0.1
7	3.2	1.2	0.5	0.1	0.4	1.0	3.3	1.2	0.5	0.1	0.4	1.0
8	4.8	1.1	0.3	0.1	3.2	0.1	4.8	1.2	0.3	0.1	3.1	0.1
9	2.9	1.1	0.4	0.3	1.0	0.1	3.1	1.2	0.5	0.3	1.0	0.1
Total		11.6	4.6	1.3	28.1	54.4		12.5	4.7	1.3	27.6	53.9

Iodine value of triglycerides 117.4(calc. from above results 121.5)

TABLE 8

Component Glycerides (% mol.)

TG	% mol.	C	1	2	222	221	220	211	210	111	200	110	100
1	21.1	0.1	0.4	20.6	19.6	1.2	0.3						
2	18.0	0.3	5.7	12.0		17.1	0.9						
3	17.1	5.5	0.7	10.9			15.6	0.9	0.6				
4	13.3	0.7	8.2	4.4				11.1	2.1	0.1			
5	15.6	5.3	5.6	4.7					12.3	1.5	1.8		
6	3.7	0.3	3.3	0.1						3.3	0.4		
7	3.3	1.8	0.5	1.0					0.6	0.3	2.4		
8	4.8	1.5	3.2	0.1						0.6	0.3	3.9	
9	3.1	1.7	1.3	0.1							0.3	1.0	1.3
				Total	19.6	18.3	16.8	12.0	15.6	5.8	5.2	4.9	1.3

TABLE 2

MAIZE OIL

(iv)

Source of Oil J. Bibby and Sons Ltd.

Purification of Triglycerides

Recovered after neutralisation (98%); contained triglycerides (95%), diglycerides (4%) and monoglycerides (1%)

Component Esters

	16:0	18:0	18:1	18:1	18:2	18:3
Oil (% mol.)	12.6	1.8	0.3	30.0	54.3	0.5

Component Esters of Fractions obtained by T.L.C.

		Increments (% Wt.)							Increments (% mol.)					
FN	% Wt.	16:0	18:0	18:1	18:1	18:2	18:3	% mol.	16:0	18:0	18:1	18:1	18:2	18:3
1	20.5	0.3	0.1	0.1	0.9	19.3	0.9	20.4	0.3	0.1	0.1	0.9	18.2	0.6
2	30.6	0.6	0.1	0.1	5.5	18.3	-	20.4	0.7	0.1	0.1	5.4	13.1	-
3	15.2	3.7	0.9	0.3	0.9	9.4	-	15.3	4.0	0.9	0.3	0.3	9.3	-
4	12.1	0.3	-	0.1	7.3	4.2	0.2	12.0	0.3	-	0.1	7.3	4.1	0.2
5	13.9	3.4	0.6	0.1	5.1	4.7	-	14.0	3.7	0.6	0.1	5.0	4.6	-
6	5.3	0.5	0.1	0.1	4.0	0.6	-	5.3	0.5	0.1	0.1	4.0	0.6	-
7	3.8	1.8	0.4	-	0.3	1.3	-	3.8	1.9	0.4	-	0.3	1.3	-
8	5.8	1.7	0.1	0.1	3.4	0.6	-	6.0	1.8	0.1	0.1	3.4	0.6	-
9	2.7	1.1	0.3	0.1	1.0	0.2	-	2.7	1.2	0.2	0.1	1.0	0.2	-
Total		13.4	2.6	1.0	29.4	52.6	1.0		14.4	2.5	1.0	29.1	52.0	1.0

Iodine value of triglycerides 113.5 (calc. from above results 123.5)

TABLE 10

Component Glycerides (% mol.)

FN	% mol.	0	1	2	3	322	222	221	220	211	210	111	200	110	100
1	20.4	0.4	1.0	18.2	0.8	2.4	13.8	3.0	1.2						
2	20.4	0.8	6.5	13.1	--			13.5	2.4	1.5					
3	15.3	4.9	1.1	9.3	-				12.6	0.6	2.1				
4	12.0	0.3	7.4	4.1	0.3				0.3	11.1					
5	14.0	4.3	5.1	4.6	-					0.9	12.9	0.2			
6	5.3	0.6	4.1	0.6	-						1.8	3.5			
7	3.3	2.3	0.3	1.3	-						0.6	0.1	3.2		
8	6.0	1.9	3.5	0.6	-							2.1	1.3	1..	
9	2.7	1.4	1.1	0.2	-								0.6	1.2	0.9
				Total		2.4	13.8	19.5	17.1	14.1	17.4	5.9	5.6	3.3	0.9

TABLE 11

COTTONSEED OIL

(v)

Source of Seeds J. Bibby and Sons Ltd.

Extraction of Oil and Purification of Triglycerides

Oil extracted 20% (based on delinted kernels)

Recovered after neutralisation (98%); contained triglycerides (88%), diglycerides (11%) and monoglycerides (1%)

Component Esters

	14:0	16:0	18:0	16:1	18:1	18:2
Oil (% mol.)	1.1	27.3	3.1	1.4	16.7	50.4

Component Esters of Fractions obtained by T.L.C.

		Increments (% Wt.)							Increments (% mol.)					
FN	% Wt.	14:0	16:0	18:0	16:1	18:1	18:2	% mol.	14:0	16:0	18:0	16:1	18:1	18:2
1	15.3	0.1	0.3	0.1	0.1	0.7	14.0	15.0	0.1	0.3	0.1	0.1	0.7	13.7
2	11.0	0.1	0.5	0.1	0.2	3.3	6.8	10.8	0.1	0.5	0.1	0.2	3.2	6.7
3	25.8	0.3	6.9	0.7	0.3	0.7	16.9	25.8	0.3	7.4	0.7	0.3	0.7	16.4
4	4.7	0.1	0.5	0.1	0.1	2.4	1.5	4.6	0.1	0.5	0.1	0.1	2.3	1.5
5	15.5	0.2	4.2	0.4	0.4	5.2	5.1	15.6	0.2	4.5	0.4	0.5	5.0	5.0
6	16.7	0.3	8.6	0.9	0.3	1.1	5.5	17.1	0.4	9.2	0.8	0.3	1.0	5.4
7	4.5	0.1	1.3	0.2	0.2	2.3	0.4	4.5	0.1	1.4	0.2	0.2	2.2	0.4
8	5.4	0.2	2.8	0.3	0.2	1.8	0.1	5.5	0.2	3.0	0.3	0.2	1.7	0.1
9	1.1	0.1	0.5	0.1	0.1	0.2	0.1	1.1	0.1	0.5	0.1	0.1	0.2	0.1
Total		1.5	25.6	2.9	1.9	17.7	50.4		1.6	27.3	2.9	2.0	17.0	49.3

Iodine value of triglycerides 96.3 (calc. from above results 104.8)

TABLE 12

Component Glycerides (% mol.)

CN	% mol.	0	1	2	222	221	220	211	210	111	200	110	100
1	15.0	0.5	0.8	13.7	11.1	2.4	1.5						
2	10.8	0.7	3.4	6.7		3.2	2.1	1.5					
3	25.8	8.4	1.0	16.4			23.7	0.6	1.5				
4	4.6	0.7	2.4	1.5				2.5	2.1				
5	15.8	5.1	5.5	5.0					14.7	0.6	0.3		
6	17.1	10.4	1.3	5.4					1.5	0.8	14.8		
7	4.5	1.7	2.4	0.4						0.6	1.2	2.7	
8	5.5	3.5	1.9	0.1							0.3	0.6	4.0
9	1.1	0.7	0.3	0.1							0.3		0.8
				Total	11.1	9.6	27.3	4.6	19.8	2.0	16.9	3.3	5.4

TABLE 13

COTTONSEED OIL (SECOND ANALYSIS)

Component Esters

	14:0	16:0	18:0	16:1	18:1	18:2
Oil (% mol.)	1.1	27.3	3.1	1.4	16.7	50.4

Component Esters of Fractions obtained by T.L.C.

FN	% Wt.	Increments (% Wt.) 14:0	16:0	18:0	16:1	18:1	18:2	% mol.	Increments (% mol.) 14:0	16:0	18:0	16:1	18:1	18:2
1	13.7	0.1	0.5	0.1	0.2	0.3	12.5	13.4	0.1	0.5	0.1	0.2	0.3	12.2
2	11.5	0.1	0.4	0.1	0.3	3.4	7.2	11.3	0.1	0.5	0.1	0.3	3.3	7.0
3	25.2	0.3	7.0	0.7	0.3	0.5	16.4	25.3	0.3	7.5	0.7	0.3	0.5	16.0
4	4.3	0.1	0.4	0.2	0.2	2.1	1.3	4.2	0.1	0.4	0.2	0.2	2.0	1.3
5	16.7	0.2	4.3	0.5	0.6	5.7	5.4	16.7	0.2	4.6	0.5	0.6	5.5	5.3
6	8.0	0.4	8.0	2.4	0.6	1.3	5.3	18.3	0.4	8.5	2.3	0.6	1.3	5.2
7	4.2	0.1	1.2	0.2	0.2	2.0	0.5	4.2	0.1	1.3	0.2	0.2	1.9	0.5
8	5.5	0.2	2.7	0.4	0.2	1.8	0.2	5.7	0.2	3.0	0.4	0.2	1.7	0.2
9	0.8	0.1	0.3	0.1	0.1	0.2	0.1	0.9	0.1	0.3	0.1	0.1	0.2	0.1
Total		1.6	24.8	4.7	2.7	17.3	48.8		1.6	26.6	4.6	2.7	16.7	47.8

Iodine value of triglycerides 96.3 (calc. from above results 102.4)

TABLE 14

Component Glycerides (% mol.) (Second Analysis)

ZN	% mol.	0	1	2	222	221	220	211	210	111	200	110	100
1	13.4	0.7	0.5	12.2	9.8	1.5	2.1						
2	11.3	0.7	3.6	7.0		7.7	2.1	1.5					
3	25.3	3.5	0.8	16.0			22.9		2.4				
4	4.2	0.7	2.2	1.3				1.8	2.1	0.3			
5	16.7	5.3	3.1	5.3					15.5	0.9	0.3		
6	18.3	11.2	1.9	5.2						0.3	15.6	2.4	
7	4.2	1.6	2.1	0.5						0.6	1.5	2.1	
8	5.7	3.6	1.9	0.2							0.6	0.6	4.5
9	0.9	0.5	0.3	0.1							0.3	0.3	0.3
				Total	9.8	9.2	27.1	3.3	20.0	2.1	18.3	5.4	4.8

TABLE 15

(vi)

GROUNDNUT OIL

Source of Seeds J. Bibby and Sons Ltd.

Extraction of Oil and Purification of Triglycerides

Oil extracted 50% (based on kernels)

Recovered after neutralisation (99%); contained triglycerides (93%), diglycerides (7%) and traces of monoglycerides.

Component Esters

	16:0	18:0	20:0	22:0	24:0	16:1	18:1	18:2	20:1	22:1
Oil (% mol.)	9.8	3.7	1.4	2.7	1.4	0.4	60.9	18.1	1.3	0.3

Component Esters of Fractions obtained by T.L.C.

		Increments (% Wt.)									
FN	% Wt.	16:0	18:0	20:0	22:0	24:0	16:1	18:1	18:2	20:1	22:1
1	1.1	0.1	0.1	-	-	-	-	0.2	0.7	-	-
2	6.4	0.1	0.1	-	-	-	0.1	2.1	3.0	-	-
3	3.4	0.7	-	-	-	-	0.1	0.4	2.2	-	-
4	19.2	0.3	-	-	-	-	0.1	12.1	6.5	0.2	-
5	12.8	2.2	0.8	0.2	-	-	-	5.4	4.1	0.1	-
6	29.6	1.0	0.2	-	-	-	0.1	26.8	1.5	-	-
7	21.9	3.8	1.9	0.1	-	-	0.2	14.8	0.6	0.5	-
8	5.2	1.8	0.8	0.1	-	-	0.1	2.3	0.1	-	-
9	0.4	0.2	-	-	-	-	-	0.1	0.1	-	-
	Total	10.2	3.9	0.4	-	-	0.7	64.2	18.8	0.8	-

Iodine value of triglycerides 80.4 (calc. from above results 86.1)

TABLE 16

Increments (% Wt. corrected)*

FN	% Wt.	16:0	18:0	20:0	22:0	24:0	16:1	18:1	18:2	20:1	22:1
1	1.1	0.1	0.1	-	0.1	-	-	0.2	0.6	-	-
2	6.2	0.1	0.1	0.1	0.1	-	0.1	2.0	3.7	-	-
3	3.2	0.7	-	-	-	-	0.1	0.3	2.1	-	-
4	18.0	0.3	-	-	-	-	0.1	11.3	6.0	0.2	0.1
5	13.2	2.1	0.8	0.3	0.7	0.3	-	5.1	3.8	0.1	-
6	27.9	1.0	0.2	0.1	0.1	0.1	0.1	25.0	1.3	-	-
7	23.7	3.6	1.8	0.7	1.6	0.9	0.2	13.8	0.5	0.5	0.1
8	6.3	1.6	0.7	0.3	0.6	0.3	0.1	2.6	0.1	-	-
9	0.4	0.2	-	-	-	-	-	0.1	0.1	-	-
Total		9.7	3.7	1.5	3.2	1.6	0.7	60.4	18.2	0.8	0.2

* Adjustment for C20:0 1.6/3.7 = 0.43
C22:0 3.2/3.7 = 0.81
C24:0 1.8/3.7 = 0.49
C22:1 .32/1.4 = 0.23

See discussion, page 103

Increments (% mol. corrected)

FN	% mol.	16:0	18:0	20:0	22:0	24:0	16:1	18:1	18:2	20:1	22:1
1	1.1	0.1	0.1	-	0.1	-	-	0.2	0.6	-	-
2	6.2	0.1	0.1	0.1	0.1	-	0.1	1.9	3.8	-	-
3	3.3	0.8	-	-	-	-	0.1	0.3	2.1	-	-
4	18.1	0.3	-	-	-	-	0.1	11.3	6.1	0.2	0.1
5	13.3	2.2	0.8	0.3	0.6	0.3	-	5.1	3.9	0.1	-
6	28.1	1.1	0.2	0.1	0.1	0.1	0.1	25.0	1.4	-	-
7	23.5	3.9	1.8	0.7	1.3	0.7	0.2	13.8	0.5	0.5	0.1
8	6.0	1.8	0.8	0.3	0.6	0.2	0.1	2.1	0.1	-	-
9	0.4	0.2	-	-	-	-	-	0.1	0.1	-	-
Total		10.5	2.8	1.5	2.8	1.3	0.7	59.8	18.6	0.8	0.2

TABLE 17

Component Glycerides (% mol.)

FN	% mol.	O	1	2	E	222	221	220	211	21E	210	20E	111	200	110	10E	100
1	1.1	0.3	0.2	0.6	-		0.4	0.7[+]									
2	6.2	0.4	2.0	3.8	-		3.7	1.2	1.3								
3	3.3	0.8	0.4	2.1	-		0.6	2.4	0.3								
4	18.1	0.3	11.4	6.1	0.3			0.3	16.3	0.9[++]	0.6						
5	13.3	4.2	5.1	3.9	0.1						10.5	0.3[*]	1.6	0.9			
6	28.1	1.6	25.1	1.4	-						3.3	-	24.0	0.8			
7	23.5	8.4	13.0	0.5	0.6									1.5	19.3	1.8[**]	0.3
8	3.0	3.7	2.2	0.1	-									0.3	0.9	-	4.8
9	0.4	0.2	0.1	0.1	-									0.2			0.2
				Total			4.7	4.6	17.9	0.9	14.4	0.3	25.6	3.7	20.8	1.8	5.3

[+] Assumed to be mixture of 220 and 221 in prop. 3:2.

[++] This fraction is mainly 211 glycerides ∴ all E assumed to be 21E.

[*] This fraction is mainly 210 glycerides ∴ all E assumed to be 20E.

[**] This fraction is mainly 110 glycerides ∴ all E assumed to be 10E.

TABLE 18

(vii)

MACADAMIA TERNIFOLIA SEED OIL

Source of Seeds — Dr. C.Y. Hopkins (Ottawa, Canada)

Extraction of Oil and Purification of Triglycerides

Oil extracted — 74% (based on kernels)

Recovered after neutralisation (99%); contained triglycerides (95%), diglycerides (4%) and monoglycerides (1%).

Component Esters

	14:0	16:0	18:0	20:0	16:1	18:1	18:2	20:1
Oil (% mol.)	0.7	9.3	3.7	2.4	27.2	51.9	2.8	2.0

Component Esters of Fractions obtained by T.L.C.

		Increments (% Wt.)							
FN	% Wt.	14:0	16:0	18:0	20:0	16:1	18:1	18:2	20:1
1	1.5	-	0.2	-	-	0.3	0.2	0.8	-
2	3.7	-	0.2	-	-	1.0	1.5	1.0	-
3	2.1	-	0.3	-	-	0.5	0.7	0.6	-
4	47.0	0.1	0.6	-	1.1	15.3	29.0	0.8	0.1
5	36.1	0.4	5.4	2.1	1.5	8.1	17.7	Tr	0.9
6	8.9	0.2	2.5	1.1	0.6	1.3	3.2	-	-
7	0.7	-	0.3	0.1	-	0.1	0.2	-	-
Total		0.7	9.5	3.3	3.2	26.6	52.5	3.2	1.0

Iodine value of triglycerides 75.3 (calc. from above results 77.1)

TABLE 19

Increments (% mol.)

FN	% mol.	14:0	16:0	18:0	20:0	16:1	18:1	18:2	20:1
1	1.5	-	0.2	-	-	0.3	0.2	0.6	-
2	3.7	-	0.2	-	-	1.0	1.5	1.0	-
3	2.1	-	0.3	-	-	0.5	0.7	0.6	-
4	47.1	0.1	0.6	-	1.0	16.4	28.1	0.8	0.1
5	36.0	0.4	5.7	2.0	1.3	8.7	17.1	-	0.8
6	8.9	0.2	2.7	1.1	0.5	1.3	3.1	-	-
7	0.7	-	0.3	0.1	-	0.1	0.2	-	-
	Total	0.7	10.0	3.2	2.8	28.3	50.9	3.2	0.9

Component Glycerides (% mol.)

FN	% mol.	0	1	2	222	221	220	211	210	111	200	110	100
1	1.5	0.2	0.5	0.8		0.3	0.6	0.6					
2	3.7	0.2	2.5	1.0				2.4	0.6	0.7			
3	2.1	0.3	1.2	0.6				0.9	0.9	0.3			
4	47.1	1.7	44.6	0.8					2.4	42.0		2.7	
5	36.0	9.4	26.6	-						7.4		28.6	
6	8.9	4.5	4.4	-								4.4	4.5
7	0.7	0.4	0.3	-								0.3	0.4
	Total					0.3	0.6	3.9	3.9	50.4	-	36.0	4.9

TABLE 20

(viii) MADHUCA LATIFOLIA SEED OIL

Source of Seeds Tropical Products Institute

Extraction of Oil and Purification of Triglycerides

Oil extracted 46% (based on kernels)

Recovered after neutralisation (98%); contained triglycerides (96%), diglycerides (3%) and monoglycerides (1%)

Component Esters

	16:0	18:0	16:1	18:1	18:2
Oil (% mol.)	23.7	24.1	0.2	37.6	14.4

Component Esters of Fractions obtained by T.L.C.

	Increments (% Wt.)						Increments (% mol.)					
FN	% Wt.	16:0	18:0	16:1	18:1	18:2	% mol.	16:0	18:0	16:1	18:1	18:2
1	0.9	0.2	0.1	-	0.2	0.4	0.9	0.2	0.1	-	0.2	0.4
2	2.5	0.3	0.2	-	0.9	1.1	2.5	0.3	0.2	-	0.9	1.1
3	3.3	0.8	0.8	-	0.5	1.7	3.3	0.8	0.8	-	0.5	1.7
4	3.8	0.3	0.2	-	2.1	1.2	3.8	0.3	0.2	-	2.1	1.2
5	13.8	2.1	2.3	0.1	5.3	4.0	13.7	2.3	2.2	0.1	5.2	3.9
6	8.0	1.5	1.1	0.1	4.3	1.0	8.0	1.6	1.1	0.1	4.2	1.0
7	34.3	6.5	10.0	0.2	13.5	4.0	34.2	7.2	9.7	0.2	13.2	3.9
8	30.7	8.8	10.8	0.2	10.9	-	30.9	8.5	10.5	0.2	10.7	-
9	2.2	0.8	1.1	-	0.2	0.1	2.2	0.9	1.0	-	0.2	0.1
Total		21.4	26.6	0.6	37.9	13.5		23.1	25.8	0.6	37.2	13.3

Iodine value of triglycerides 58.0 (calc. from above results 59.1)

TABLE 21

Component Glycerides (% mol.)

FN	% mol.	0	1	2	222	221	220	211	210	111	200	110	100	000
1	0.9	0.3	0.2	0.4			0.3		0.6					
2	2.5	0.5	0.9	1.1			0.6	0.9	1.0					
3	3.8	1.6	0.5	1.7			2.1		1.7					
4	3.8	0.5	2.1	1.2				2.1	1.5	0.2				
5	13.7	4.5	5.3	3.9					10.2	1.9	1.6			
6	8.0	2.7	4.3	1.0						2.9	3.0	2.1		
7	34.2	16.9	13.4	3.9							11.7	17.7	4.8	
8	30.9	20.0	10.9	-								1.8	29.1	
9	2.2	1.9	0.2	0.1							0.3		0.6	1.3
Total							3.0	3.0	15.0	5.0	16.6	21.6	34.5	1.3

TABLE 22

(ix) **GMELINA ASIATICA SEED OIL**

Source of Seeds — Singapore

Extraction of Oil and Purification of Triglycerides

Oil extracted — 59% (based on kernels)

Recovered after neutralisation (98%); contained triglycerides (98%) diglycerides (2%) and traces of monoglycerides.

Component Esters

	16:0	18:0	20:0	22:0	16:1	18:1	18:2	20:1	22:1
Oil (% mol.)	10.1	8.1	2.6	2.4	0.3	28.8	37.6	9.9	0.2

Component Esters of Fractions obtained by T.L.C.

		Increments (% Wt.)								
FN	% mol.	16:0	18:0	20:0	22:0	16:1	18:1	18:2	20:1	22:1
1	6.7	-	-	0.1	-	-	0.2	6.3	T_r	0.1
2	16.6	0.2	0.1	-	-	-	3.5	10.7	2.0	0.1
3	12.1	1.3	1.2	0.6	0.5	-	0.6	7.6	0.2	0.1
4	8.0	0.1	-	-	-	-	5.0	2.7	0.2	-
5	6.8	0.1	0.1	-	-	-	2.6	2.6	1.4	-
6	12.7	1.9	1.3	0.5	0.2	0.1	4.2	4.2	0.3	-
7	11.0	0.9	0.9	0.3	0.3	0.1	4.0	2.5	2.0	-
8	4.6	0.6	0.2	-	-	-	2.0	0.6	1.2	-
9	7.4	1.5	1.6	0.5	0.3	0.1	0.7	2.2	0.5	-
10	9.6	1.3	1.1	0.4	0.3	0.1	5.0	0.2	1.2	-
11	4.2	1.1	1.1	0.3	-	0.1	1.6	-	-	-
12	0.3	0.1	0.1	-	-	-	0.1	-	-	-
Total		9.1	7.7	2.7	1.6	0.5	29.5	39.6	9.0	0.3

Iodine value of triglycerides 96.4(calc. from above results 97.6)

FN	% mol.	16:0
1	6.8	-
2	16.6	0.2
3	12.1	1.4
4	8.1	0.1
5	6.7	0.1
6	12.8	2.1
7	10.8	1.0
8	4.5	0.7
9	7.4	1.7
10	9.6	1.4
11	4.3	1.2
12	0.3	0.1
Total		10.0

TABLE 23

Increments (% mol)

18:0	20:0	22:0	16:1	18:1	18:2	20:1	22:1
-	0.1	-	-	0.2	6.4	Tr	0.1
0.1	-	-	-	3.5	10.8	1.9	0.1
1.2	0.6	0.4	-	0.6	7.6	0.2	0.1
-	-	-	-	5.0	2.8	0.2	-
0.1	-	-	-	2.6	2.6	1.3	-
1.3	0.6	0.2	0.1	4.2	4.2	0.6	-
0.9	0.3	0.2	0.1	3.8	2.5	1.9	-
0.2	-	-	-	2.0	0.5	1.1	-
1.5	0.5	0.3	0.1	0.7	2.1	0.5	-
1.1	0.4	0.3	0.1	5.0	0.2	1.1	-
1.1	0.3	-	0.1	1.6	-	-	-
0.1	-	-	-	0.1	-	-	-
7.6	2.6	1.4	0.5	29.4	39.7	8.5	0.3

TABLE 24

Component Glycerides (% mol.)*

FN	% mol.	O	1	2	E	222	221	22E	220	211	21E	210	2EO	111	11E	200	11C	1EO	100
1	6.8	0.1	0.2	6.4	0.1	5.6	0.6	0.3	0.3										
2	16.6	0.3	3.5	10.8	2.0		9.0	6.0	0.9	0.7									
3	12.1	3.6	0.6	7.6	0.3				10.6	0.3	0.9	0.3							
4	8.0	0.1	5.0	2.8	0.2				0.3	7.2	0.6								
5	6.7	0.2	2.6	2.6	1.3				0.6	2.2	3.9								
6	12.8	4.0	4.3	4.2	0.3						0.9	11.9							
7	10.8	2.4	4.0	2.5	1.9						0.3	1.8	5.4	3.3					
8	4.5	0.9	2.0	0.5	1.1								0,9	0.3	2.4	0.9			
9	7.4	4.0	0.8	2.1	0.5								0.6	0.2	0.9	5.7			
10	9.6	3.2	5.1	0.2	1.1								0.4				6.2	3.0	
11	4.3	2.6	1.7	-	-												0.6		3.7
12	0.3	0.2	0.1	-	-														0.3
Total						5.6	9.6	6.3	12.7	10.4	6.6	14.0	7.3	3.8	3.3	6.6	6.8	3.0	4.0

* The results are calc. on the following assumptions

(i) OCO,COE,OEE and EEE (equivalent to [illegible]) are taken to be absent.
(ii) 1EE and 2EE are assumed to be absent.

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PART III

Partial Hydrolysis with Pancreatic Lipase

PART III

Partial Hydrolysis with Pancreatic Lipase

1. Discussion

1. Methods

The specificity of pancreatic lipase has been studied by a number of workers. Thus Desnuelle et al.[1-3] and Mattson et. al.[4-7] have shown that pancreatic lipase hydrolyses the $C_{(1)}$ and $C_{(3)}$ positions of triglycerides, a reaction which provides valuable information about glyceride structure. The studies were further extended by Mattson and Volphenhein[8,9] who, from a study of vegetable fats, concluded that $C_{(2)}$ glycerol hydroxyl group is preferentially acylated with C_{18} unsaturated acids (Category 1 acids) and $C_{(1)}$ and $C_{(3)}$ hydroxyl groups are subsequently acylated with saturated and C_{20} and C_{22} unsaturated acids and with those C18 unsaturated acids not required at $C_{(2)}$ position (Category 11, acids). This theory of acyl group distribution has been accepted by Vander Wal[10] and Coleman[11], who calculated the glyceride composition of vegetable fats from their lipolysis results on the assumption that the acids liberated by pancreatic lipase are randomly distributed at $C_{(1)}$ and $C_{(3)}$ positions. We have now examined a number of vegetable fats both by chromatographic procedures and lipolysis and confirmed and extended the above theory of acyl group distribution.

The oils were subjected to pancreatic lipase which preferentially removed the fatty acids from $C_{(1)}$ and $C_{(3)}$ positions of triglycerides. The monoglycerides were isolated either by column or thin layer chromatography. The latter method is quicker and more effective. In this method the products of lipolysis were applied to silica gel plates and developed with a mixture of chloroform and acetone (80 ml:20 ml.) to which ammonia (1.5 ml.) was added to hold back the free fatty acids which otherwise travelled along with triglycerides. The other components, monoglycerides, diglycerides and triglycerides, travelled according to their decreasing polarity. More recently, a similar method of separating lipolysis products by thin layer chromatography has been described by Luddy et al.[12] who used a mixture of ether-chloroform (40:60) containing 1.6 per cent formic acid as developing solvents.

The zones were detected, after spraying with dichlorofluorescein, under ultraviolet light. The monoglycerides were extracted from the adsorbent and after transesterification the component esters were determined by gas liquid chromatography. The two methods (column and thin layer chromatography) gave similar results.

From the composition of the triglycerides and the 2-monoglycerides the component glycerides of seed oils (safflower, tobacco, J. gossypifolia, sunflower, J. multifida, A. mexicana, maize, cotton, groundnut, M. ternifolia, G. asiatica, J. curcas and M. latifolia) were calculated

on the assumption that acids at the $C_{(2)}$ position and acids at the $C_{(1)}$ and $C_{(3)}$ positions are statistically distributed thereat. The value of all the possible glycerides, including isomers, were calculated and then combined in the main categories determined by the analytical procedures reported in Parts I and II. The value shown against 210 glyceride, for example, is the total value for the six triglycerides containing one linoleic acid group, one oleic acid group and one palmitic or stearic acid group. The results agree with those obtained by chromatographic procedures (Parts I and II) or calculated directly from the component esters on the basis of a theory of positional distribution proposed by Gunstone[13].

The distribution of lauric, myristic, hexadecencic, oleic, linoleic and eicosenoic acids in vegetable seed oils was also studied. The results, discussed in terms of selectivity factor, indicate that unsaturated acids do not compete equally for the secondary hydroxyl group.

2. Lipolysis Results

Mattson and Volpenhein,[8,9] from an examination of the distribution of C18 unsaturated acids, concluded that there is a slight tendency for more linoleic than oleic to be present in the $C_{(2)}$ position than would be expected from their proportion in the whole oil. Except for these differences, C18 unsaturated acids (Category II) are randomly distributed among the positions in the triglyceride

molecules that are not occupied by saturated acids and C20 and C22 unsaturated acids (Category I). We confirmed the preference of linoleic acid over oleic acid for the $C_{(2)}$ position and extended this observation in terms of an "enrichment factor"

We use the concept of an "enrichment factor" to discuss the acyl group distribution. The enrichment factor is the ratio of the concentration (molar) of an acid group in the 2 position to its concentration in the total glycerides. The enrichment factor can have any value between 0 and 3; values below 1 indicate a preference of an acid for $C_{(1)}$ and $C_{(3)}$ positions, values above 1 indicate a preference for $C_{(2)}$ position. This enrichment factor is useful when comparing values for acids competing for the $C_{(2)}$ position in the same fat but it is less convenient in discussing the behaviour of acids in several different fats and for this reason we have used another term: the "selectivity factor". This is defined as the ratio of the enrichment factor of a particular acid divided by the enrichment factor for all the Category II acids present in the fat under consideration.

This is illustrated by the values for safflower oil Table 12, Page 166. The enrichment factors are obtained for each acid by dividing values in the second row of figures (2-monoglycerides) by the appropriate values in the first row (triglycerides). The selectivity factors of the Category II acids are then obtained by dividing the enrichment factor for each acid by the enrichment factor obtained for the total group of Category II acids.

Oleic and Linoleic Acids

The value of the selectivity factor is quite apparent from Table 1, page 135, where information on about 40 seed oils is given. Linoleic acid almost always has a selectivity factor greater than oleic acid so that the chance of linoleic acid being present in the $C_{(2)}$ position is slightly higher and that of oleic acid slightly lower than expected. These results confirm and extend the views of Mattson and Volphenhein.[9]

TABLE 1a

Selectivity factors for Oils containing hexadecenoic acid

		Component Pattern (% mol.)				Selectivity factors		
Ref.	Name	"sat."	16:1	18:1	18:2	16:1	18:1	18:2
9	Avocado	37	20	27	14	0.4	1.0	1.8
Q	Macadamia	18	27	52	3	0.7	1.1	-
	ternifolia	23	18	56	3	0.7	1.1	-
Q	Gevuina	24	24	44	8	0.8	1.0	1.5
	avellana	25	24	42	9	0.8	1.0	1.6

TABLE 1 (Continued)

Selectivity factors for Oils containing Oleic and Linoleic acids

Ref.	Name	Component Esters (% mol.)			Selectivity factors	
		"sat"	18:1	18:2	18:1	18:2
Q	Sunflower	7	50	42	0.9	1.1
9	Filbert	9	82	8	1.0	1.4
9	Almond	9	70	21	0.3	1.5
9	Sunflower	9	27	64	0.8	1.1
9	Onion	10	26	64	0.9	1.0
Q	Safflower	10	13	77	0.9	1.0
Q	Sunflower	10	30	60	0.9	1.1
9	Acorn	10	69	20	0.9	1.4
9	Safflower	11	14	75	0.8	1.0
Q	Sunflower	12	26	62	0.8	1.1
9	Pecan	12	58	30	0.9	1.2
9	Poppy	12	11	76	0.7	1.0
9	Spinach	13	24	60	0.8	1.1
Q	Tobacco	14	14	71	1.0	1.0
Q	*J. gossypifolia*	14	17	69	1.0	1.0
9	Corn	14	29	53	0.8	1.1
9	Olive	14	76	8	1.0	1.1
Q	Maize	15	30	54	0.9	1.1
15	Olive	15	74	10	1.0	1.0
14	Olive	15	74	11	1.0	0.8
9	Tomato	16	21	61	0.9	1.0
9	Sesame	16	40	44	0.9	1.1
Q	*A. mexicana*	17	28	55	1.1	1.0
8	Peanut	18	52	27	0.8	1.4
9	Rice	19	41	38	0.9	1.2
Q	Groundnut	19	61	18	0.9	1.4
Q	*J. curcas*	20	40	37	0.8	1.2
9	cucumber	21	7	71	0.5	1.1

TABLE 1 (Continued)

Ref.	Name	Component Esters (% mol.) "sat."	18:1	18:2	Selectivity factors 18:1	18:2
15	Peanut	21	50	29	0.9	1.2
9	Cashew	22	60	18	0.7	1.9
8	Morro	24	51	22	0.9	1.2
9	Squash	24	16	60	0.8	1.1
Q	J. multifida	26	23	49	0.8	1.1
8	Marigold	28	9	62	0.8	1.0
9	Cottonseed	28	17	55	0.9	1.0
9	Brazil nut	29	33	39	0.9	1.1
15	Cottonseed	30	19	51	0.8	1.1
Q	Cottonseed	32	18	50	1.1	1.0
16	Illipe	42	53	5	1.0	0.7
8	Coffee	44	7	48	0.8	1.0
17	Shea	46	47	7	1.0	1.3
16	Palm	46	46	8	1.0	1.2
Q	M. latifolia	48	38	14	0.9	1.3
15	Palm	49	40	11	1.0	0.9
9	Palm	50	39	9	1.0	1.4
16	Karite	50	45	5	1.0	1.4
17	Palm	51	42	7	1.0	1.1
15	Shea	52	41	6	1.0	1.3

	Selectivity factor <1.0	1.0	>1.0	The larger value
Oleic	32	15	2	5 times
Linoleic	3	14	32	41 times

Hexadecenoic Acids

Hexadecenoic acid is most abundant in fats of aquatic origin and rarely exceeds 1 or 2% of the component acids in vegetable fats. It is, however, a major component of avocado pear, M. ternifolia and G. avellana seed oils.

The Hexadecenoic acids of G. avellana and M. ternifolia were also examined qualitatively and our results confirmed those previously reported by Cattaneo et al.[18] and Hilditch, et al.[19] The C16 acids are not the same, the Δ^{11} isomer is present in G. avellana and the Δ^{9} isomer in M. ternifolia seed oil.

These two acids were isolated by a combination of preparative gas liquid chromatography and silica-silver nitrate column chromatography. When subjected to periodate-permanganate oxidation[20] they gave undecanodioic acid and azelaic acid respectively.

The results for the avocado pear are those reported by Mattson and Volpenhein[9]. We have examined two samples of M. ternifolia seed oil and two of G. avellana seed oil (Table 12, Page 166). The behaviour of oleic and linoleic acid is normal with the linoleic acid having a higher selectivity factor than oleic acid. Hexadecenoic acid, whether the Δ^{9} acid in avocado pear or M. ternifolia or the Δ^{11} acid in G. avellana, appears in the $C_{(2)}$ position but its low selectivity factor shows that it is less

likely to be found there than either of the C18 unsaturated acids. See Table 1a (P. 134)

Eicosenoic Acid

The component acids of C. halicacabum were reported by Hopkins et. al.[21] and we have now confirmed their results that eicosenoic acid (40%) is the major component of the oil with only 13% of arachidic acid. The behaviour of oleic and linoleic acids in the 2- monoglycerides is normal as there is less oleic acid and more linoleic acid than would be expected. Again, there is some evidence that eicosenoic acid which constitutes 17% of 2-monoglyceride is not exclusively distributed at the $C_{(1)}$ and $C_{(3)}$ positions as shown by Mattson and Volpenhein[8] in other vegetable seed oils containing this acid.

Lauric and Myristic Acids

Three seed oils (Irrvingia gabonensis, Pycanthus angolensis and Myristica malabarica) containing various amounts of myristic and lauric acids were examined. The composition of P. angolensis has not been reported previously. Moreover, our analysis of I. gabonensis and M. malabarica differ from those obtained by fractional distillation.[22,23]

It seems from our lipolysis results (see Table 12, page 166) that lauric and myristic acids do not behave as typical saturated acids and this has also been reported previously.[47] It is quite

apparent from the enrichment factor of myristic acid (40%, 1.12) and oleic acid (28%, 1.11) in M. malabarica fat that these two compete equally for the $C_{(2)}$ position. The distribution of lauric and myristic acids in I. gabonensis and P. angolensis seed fats is not clear. In I. gabonensis fat the myristic acid (48%, enrichment factor 1.56) competes more successfully for the $C_{(2)}$ position then lauric acid (34%, "enrichment factor" 0.43), where in P. angolensis fat the lauric acid (12%, "enrichment factor" 1.73) is more likely to be in $C_{(2)}$ position than the myristic acid (75%, enrichment factor 0.87). Further evidence from seed oils of this type is required before any clear pattern can be discerned

3. The Component Glycerides of Oils containing Saturated, Oleic and Linoleic Acids

We have divided the oils into four groups according to the proportion of linoleic acid, oleic acid and saturated acids.

(i) The three J. oils (Jatropha curcas, J. multifida and J. gossypifolia) which we examined by crystallisation and column chromatography are all considered into one group irrespective of their fatty acids proportion.

(ii) Oils with a very high proportion of linoleic acid (> 70%); safflower and tobacco.

(iii) Oils with a high proportion of linoleic acid (50-60%) sunflower, Argemone mexicana, maize and cottonseed.

(iv) Oils with a high proportion of oleic acid (> 50%); groundnut, Macadamia ternifolia.

(v) Oils with a high content of "saturated acids" (> 30%) Madhuca latifolia (Mowrah butter) and Gmelina asiatica.

Some of the oils fall into more than one group but we have chosen to include them in the group given above rather than in another.

(i) Jatropha Oils

Information about Jatropha seed oils, previously limited to the component acids of J. curcas,[24] J. glandulifera,[25] and J. macrocarpa,[26] is now extended to two further species: J. multifida and J. gossypifolia. The results are summarised below:-

Component Esters (% wt.)

	J. curcas	J. glandulifera	J. multifida	J. macrocarpa	J. gossypifolia
16:0 + 18:0	21.9	22.8	24.6	15.2	12.8
16:1 + 18:1	41.5	34.2	25.0	33.4	16.6
18:2	36.6	43.0	50.4	51.4	70.6

The Jatropha seed oils are examined by crystallisation and column chromatography and it is worth noting that our results differ from those of seed oils of similar composition previously examined

by the normal low crystallisation processess. The major glycerides for J. multifida seed oil are compared with those of Chorozophora plicata[27] and cottonseed oils[28] and those for J. gossypifolia are compared with results for tobacco seed oil and sunflower seed oil. Though differing from these our results are similar to those calculated from lipolysis data (L) or derived from component acids according to the theory elaborated by Gunstone. (see Table 2, Column P).

Component Esters (% mol.)

	(a)	(b)	(c)	(d)	(e)	(f)
Saturated (O)	23	29	26	15	11	14
Oleic (1)	25	24	25	18	17	17
Linoleic (2)	52	47	49	67	72	69

Component Glycerides (% mol.)

210	28	41	22	6	3	8
220	19	18	23	39	23	26
211	-	-	9	-	-	6
221	37	28	15	47	48	22
222	3	-	10	8	23	33

(a) Chorozophora plicata[27] (b) cottonseed[28] (c) J. curcas[Q]
(d) sunflower[29] (e) tobacco[30] (f) J. gossypifolia[Q]

TABLE 2

Component Esters and Glycerides (% mol.) of Jatropha Oils

Component Esters

	J. curcas	J. multifida	J. gossypifolia
16:0 + 18:0	22	25	13
16:1 + 18:1	41	25	17
18:2	37	50	70

Component Glycerides

	A	L	T	A	L	T	A	L	T
S_2U	10	9	10	15	15	15	3	4	4
SU_2	46	41	44	51	47	47	35	32	32
U_3	44	50	46	34	38	38	62	64	64
222	4	5	5	9	10	11	33	33	33
221	17	17	16	15	17	17	22	24	24
220	10	9	10	23	21	21	26	21	21
211	15	20	18	9	9	9	6	6	6
210	24	21	22	23	21	21	8	10	10
111	8	8	7	1	2	1	1	1	1
200	7	5	5	11	11	10	2	3	3
110	12	11	12	5	5	5	1	1	1
100	3	4	5	4	4	5	1	1	1

A Crystallisation and chromatography

L Lipolysis

T Calculated according theory (Gunstone)

TABLE 3

Component Ester and Glycerides (% mol.) of Safflower and Tobacco seed oils

Ref. + Methods*	Safflower			Tobacco	
Component Esters	C^{29}	D^{31}	Q	C^{30}	Q
16:0 + 18:0	11	11	10	11	14
16:1 + 18:1	13	13	13	17	14
18:2 + 18:3	76	76	77	72	72

Component Glycerides

			A	L	T+		A	L	T
S_2U			2	2	2	3	7	5	4
SU_2	32		26	26	26	27	33	32	33
U_3	68		72	72	72	70	63	63	63
322	-	-	-	-	-	-	2	2	2
222	31	47	47	45	45	19	33	35	34
221	37	36	19	23	23	51	17	22	22
220	30	8	13	19	19	23	24	22	23
211	-	-	5	4	4	-	5	4	5
210	2	-	7	6	6	4	8	9	9
111	-	-	1	-	-	-	3	-	-
200	-	-	1	2	2	3	7	4	3
110	-	-	2	1	1	-	1	1	1
100	-	-	-	-	-	-		1	1

*These methods refer to crystallisatior (C), countercurrent distribution (D), thin layer argentation (A), gas liquid chromatography (G), oxidation (O), lipolysis (L) and reverse phase chromatography (R). "Q" present work.

(ii) Safflower and Tobacco

The glyceride composition of these oils, both of which contain over 70% of linoleic acid, is given in Table 3, along with results of Barker and Hilditch,[29] Scholfield et al.[31] and Crawford and Hilditch.[30] The high content of linoleic is reflected in the proportion of 222 (47% and 33%) and 221 glycerides (37% and 43%). There is a good agreement between our own results obtained by three different ways. Our results are also similar to those obtained by Scholfield et al.[31] by countercurrent distribution. However, they do not show good agreement, particularly in respect of individual glycerides, with earlier results obtained by low temperature crystallisation. This is not surprising as we have proved in Part I that this method is not suitable for oils containing appreciable quantity of more than one unsaturated acids.

(iii) Sunflower, Argemone mexicana, Maize and Cottonseed

Sunflower: The results of these seed oils are quoted, along with previous results, where relevant, in Table 4 page 145. We had three samples of sunflower seed oils (two Nigerian varieties and one Bulgarian variety) two of which possessed a similar fatty acid composition (see Table 12). All the three oils were examined by lipolysis but only two by low temperature crystallisation. Though only two of the three samples contain high linoleic acid content (60 and 62%) it is convenient to consider all together.

TABLE 4

Component Esters and Glycerides (% mol.) of Sunflower seed oils.

Ref. + Methods*	C^{29}	Q		Q		A^{48}	C^{29}	C^{29}	Q		C^{32}
Component Esters											
16:0 + 18:0	13	12		10		14	11	12	7		10
16:1 + 18:1	24	26		30		28	33	44	51		49
18:2	63	62		60		58	56	44	42		41
Component Glycerides		L	T+	A	L	T				A	L	T	
S_2U	2	3	3	2	3	3	-	-	1	1	2	1	-
SU_2	35	28	28	26	35	25	-	32	34	22	18	19	31
U_3	63	79	79	72	72	72	-	68	65	77	80	80	69
222	7	23	24	14	20	21	19			4	7	7	-
221	56	31	30	39	32	32	28	61	39	31	27	27	24
220	20	14	14	14	11	11	14	7		7	4	4	-
211	-	13	13	19	17	16	14	7	26	29	33	33	45
210	15	12	12	11	11	11	12	25	27	11	9	9	31
111	-	2	2	-	3	3	6	-		13	13	13	-
200	2	2	2	1	2	2	5	-		-	1	1	-
110	-	2	2	1	3	3	2	-	7	4	5	5	-
100	-	1	1	1	1	1	-	-	1	1	1	1	-

*+ Same as footnotes to Table 3.

TABLE 5

Component Esters and Glycerides (% mol.) of A. mexicana seed oil and maize oil

	A. mexicana	Maize				
Ref. + . Methods*						
Component Esters	Q	Q	C^{33}	D^{34}	O^{35}	O^{36}
16:0 + 18:0	17	14	15	13	13	16
16:1 + 18:1	28	31	24	27	27	?
18:2 + 18:3	55	55	61	60	60	?
Component Glycerides						
	A L T+	A L T				
S_2U	7 6 6	7 5 5	2	-	4	5
SU_2	38 37 37	37 33 34	41	-	34	34
U_3	55 57 57	56 62 61	57	-	62	61
322	- - -	2 1 1	-		-	
222	20 17 17	14 15 15	1	22	20	
221	18 25 25	20 27 27	49	24	26	
220	17 16 16	17 14 14	34	13	16	
211	12 13 13	14 16 15	7		14	
210	16 17 17	17 15 16	6		15	
111	5 2 2	6 3 3	-		2	
200	5 4 4	6 3 3	2		3	
110	5 4 4	3 4 4	1		3	
100	2 2 2	1 2 2	-		1	

*+ Same as footnotes to Table 3.

Our results agree with those of Kaufmann[48] but differ from older results obtained by low temperature crystallisation.[29,32]

Maize. Maize oil has been studied extensively by low temperature crystallisation,[33] countercurrent distribution[34] and by two different oxidation procedures.[35,36] Our results though different from those obtained by low temperature crystallisation are similar to those obtained in the incomplete analysis by countercurrent distribution. There is also good agreement with those obtained by two different oxidation procedures described by Privett and Blank[35] and by Subbaram and Youngs[36]. (see Table 5).

Argemone mexicana. The glyceride composition of this oil which contain 55% of linoleic acid has not been reported previously. Its component glycerides are similar to those of maize oil thus emphasising the previously accepted view that glyceride composition of a seed oil depends upon its component acids and not on its biological origin. (see Table 5).

Cottonseed. Hilditch and Maddison[28] determined the component glycerides of this oil by low temperature crystallisation and their results have been reinterpreted and recalculated by Gunstone.[13] The oil has also been examined by two different oxidation methods and by lipolysis[36,38] and Vereshchagin[37] has studied the oil by a combination of adsorption chromatography on alumina, reverse phase chromatography and gas liquid chromatography. More recently,

TABLE 6

Component Esters and Glycerides (% mol.) of Cottonseed Oil

Ref. + Methods*	Q				O[36]	C[28]	R and G[37]	O[38]	C[40]	A and L[17]		A and L[39]	
Component Esters													
16:0 + 18:0	32				33	29	27	27	23	-		30	
16:1 + 18:1	18				17	22	16	?	?	-		19	
18:2	50				50	47	57	?	?	-		51	
Component Glycerides													
	A1	A2	L	T+								A	L
S_2U	23	23	22	22	22	13	17	13	13	-		18	21
SU_2	50	52	48	50	51	59	48	48	44	-		48	49
U_3	27	25	29	28	27	28	35	39	43	-		33	30
										A	L		
222	11	10	12	11			17			6	15	13	12
221	10	9	12	12		28(18)**	14			13	13	13	13
220	27	27	26	27		18(31)	29			30	26	24	25
211	5	3	4	4		-(9)	3					6	4
210	20	20	19	19		41(26)	17			23	18	19	20
111	2	2	1	1		-(1)	-					1	1
200	17	18	16	16		7(10)	12			23	21	13	14
110	3	6	3	4		-(2)	3					5	4
100	5	5	6	6		6(3)	5			5	7	5	7
000	-	-	1	-								1	-

*+ Same as footnotes to Table 3

** Results recalculated by Gunstone[13]

Jurriens et al.[39] carried out the analysis by thin layer argentation procedure and submitted each fraction to pancreatic lipase hydrolysis. Our results agree with the more recent studies but differ from those obtained by low temperature crystallisation. Cottonseed oil was examined twice and there is a good agreement between our two sets of results. (see Table 6).

(iv) Groundnut and Macadamia ternifolia

Groundnut: These two oils are both rich in oleic acid but otherwise they have little resemblance. In groundnut oil four glycerides (see Table 7) 221, 210, 111 and 110 make up almost 80% of the total but six minor glycerides are also present. The analysis by low temperature crystallisation[41] picked out three major glycerides (210, 210 and 110, almost 80%) and three minor components. Our results are similar to, but more detailed than, those of Barrett et al.[17] obtained by the thin layer argentation procedure. More recently, a sample of groundnut oil rich in linoleic acid (41%) has been examined both by lipolysis and thin layer argentation procedure by Jurriens et al.[39]

TABLE 7

Component Esters and Glycerides (% mol.) of Groundnut Oil and G. asiatica seed oil

Ref. + Methods	Groundnut				G. asiatica		
	Q	A and L^{17}		C^{41}	C^{40}	Q	
Component Esters							
sat.	19			20	20	23	
16:1 + 18:1	61			59	-	29	
20:1 + 22:1	2			21	-	10	
18:2	18			-	-	38	
Component Glycerides							
	A	L	T			A	L	T
S_2U	11	10	9		7	9	21	24	25
SU_2	40	42	47		47	42	48	51	50
U_3	49	48	44		46	49	31	25	25
				A L					
222	-	-	1	-			6	4	5
221	5	5	6	8 3	6		10	10	10
22E	-	-	-				6	5	5
220	4	2	2				12	11	11
211	18	20	19	24 22	34		11	9	8
21E	1	1	1				6	8	7
2EE	-	-	-				-	1	1
210	14	15	14		16		14	17	17
2EO	-	-	-				7	6	6
111	26	23	22	39 47	6		4	2	2
11E	-	2	2				3	3	3
1EE	-	-	-				-	1	1
200	4	3	2				7	7	7
110	21	22	24	26 24	31		7	7	7
E10	2	1	1				3	4	5
100	5	6	6	3			4	5	5

M. ternifolia: This oil is of interest in that it contains C16 and C18 monoethenoid acids in appreciable proportions (31%) but its component glycerides have not been previously reported. Our thin layer argentation study did not distinguish glycerides in which these two acids were interchanged but the total amount in each group of similar glycerides agrees so well with those obtained by lipolysis that the figures for individual glycerides can be taken from the lipolysis results (column L, Table 8). Our results show that five major glycerides (111, 11H, 1HH, 110 and H10) comprise over 30% of the oil accompanied by ten minor components.

(v) Gmelina asiatica: The sample of G. asiatica contained 23% of saturated acids along with 10% of eicosenoic acid which behaves, in respect of acyl distribution, as a saturated acid. This content of "saturated" acids (33%) is interesting for it is at this value that the difference between widest distribution (requiring 100% of S_2U) and positional distribution (requiring 50% of S_2U) is greatest[13]. Our value of 48% is therefore highly significant. The subfractionation of oleic and eicosenoic glycerides already referred to allowed us to distinguish between their glycerides with the results shown in Table 7. The greater number of acids in this oil means more glyceride categories. Fourteen are distinguished with four (221,220,221 and 210) exceeding 10% and a further six each present to the extent of 6 to 7%.

TABLE 8

Component Esters and Glycerides (% mol.) of M. ternifolia seed oil

Component Esters

sat.	18
16:1	27
18:1 + 20:1	54
18:2	3

Component Glycerides

	A	L	T
S_2U	5	6	6
SU_2	40	36	37
U_3	55	58	57
220	1	-	-
211	)	2)	2)
21H*	) 4	3) 5	2) 5
2HH	)	1)	1)
210	)	2)	2)
2HO	) 4	1) 3	1) 3
111	)	15)	15)
11H	)50	24) 53	23) 52
1HH	)	12)	12)
HHH	)	2)	2)
200	-	-	-
110	)	15)	15)
1HO	)	15) 33	15) 34
HHO	)36	3)	4)
100	)	4)	4)
HOO	) 5	2) 6	2) 6

* H stands for hexadecenoic acid.

(vi) Maduca latifolia (Mowrah butter)

This is the most saturated fat we examined. It contains eight categories of glycerides with four of them (210, 200, 110 and 100) comprising nearly 90% of the whole fat. The study of this more saturated material shows the limitations of our thin layer argentation procedure in that it does not distinguish between the various saturated glycerides. Half of M. latifolia contains 100 and 200 glycerides but within each group we cannot distinguish between the dipalmito-, the palmito- stearo- and the distearo- monounsaturated glycerides. In this respect we consider our procedure to be complementary to that of Youngs et al.[36,38] which distinguishes between saturated but not between unsaturated acyl groups. (see Table 9).

Conclusion

The results obtained both by thin layer argentation procedure and by crystallisation and column chromatography of linoleic containing seed oils are also classified according to the number of polyethenoid acid groups present in the glyceride and the number of double bonds present in the glycerides. (see Tables 10 and 11). The seed oils (safflower, tobacco, Jatropha gossypifolia and linoleic rich sunflower) which contain 67-84% of glycerides having two or three linoleic chains are likely to show some drying properties.

TABLE 9

Component Esters and Glycerides (% mol.) M. latifolia seed oil

Ref. + Method*				C^{40}	C^{42}
Component Esters					
16:0 + 18:0		48		43	44
16:1 + 18:1		38		?	43
18:2		14		?	13
Component Glycerides					
	A	L	T		
S_2U	52	46	51	47	28
SU_2	39	41	41	36	71
U_3	8	9	8	17	-
221	-	1	1		
220	3	2	3		
211	3	4	4		
210	15	17	16		
111	5	4	3		
200	17	15	14		
110	21	22	22		
100	35	31	27		
000	1	4	-		

*Same as footnotes to Table 3.

TABLE 10

Proportions (% mol.) of polyethenoid Glycerides

	Component esters (% mol.)		Component Glycerides			
	Pe	x	Pe_3	Pe_2x	Pex_2	X_3
Safflower	77	23	47	37	14	2
Tobacco	72	28	35	41	20	4
J. gossypifolia	69	31	33	48	16	3
Sunflower	60	40	14	53	31	2
A. mexicana	55	45	20	35	33	12
Maize	55	45	16	37	36	11
Cottonseed	50	50	11	36	42	11
J. multifida	49	51	9	38	43	10
Sunflower	42	58	4	38	40	18
G. asiatica	38	62	6	28	45	21
J. curcas	37	63	3	27	46	24
Groundnut	18	82	-	9	37	54
M. latifolia	14	86	-	3	35	62
M. ternifolia	3	97	-	1	8	91

TABLE 11

Glyceride Categories (% mol.) of linoleic containing oils

	sat. acids (% mol.)	Number of double bonds						
		7 + 6	5	4	3	2	1	0
Safflower	10	47	19	23	8	3	-	-
Tobacco	14	35	17	29	11	8	-	-
J. gossypifolia	14	33	22	32	9	3	1	-
Sunflower	10	14	39	33	11	2	1	-
Maize	14	16	20	31	23	9	1	-
A. mexicana	17	20	18	29	21	10	2	-
Sunflower	7	4	31	30	24	4	1	-
J. multifida	26	10	15	32	24	15	4	-
J. curcas	20	3	16	26	32	19	4	-
Cottonseed	32	11	9	32	22	20	6	-
G. asiatica	33	6	16	29	28	17	4	-
Groundnut	21	-	5	23	40	27	5	-
M. ternifolia	16	-	-	5	55	35	5	-
M. latifolia	48	-	-	6	20	38	35	1

There is a good agreement between the results obtained by chromatographic proceedures and those calculated from our lipolysis results by the method proposed by Vander Wal[10] and by Coleman[11] or derived directly from component acids. This agreement between the results confirm the assumptions of Vander Wal and Coleman thus validating their calculations of triglycerides composition from enzyme-hydrolysis results. Moreover, the acyl group distribution theory is confirmed and thus it is possible to calculate fairly accurately the component glycerides of vegetable fats from a knowledge of their component acids. However, the theory of acyl group distribution is only applicable to seed oils containing saturated oleic, linoleic and linolenic acids and a possibility remains that some oils, especially those containing unusual acids may deviate from this pattern of distribution. An important example of this has recently been reported (Momordica charantia) by Subbaram, Chakrabarty, Youngs, and Craig.[46]

EXPERIMENTAL

Lipolysis[11]

Triglycerides (1g.), in a double walled vessel, kept at 37°C by circulating water between the two walls, were dispersed with a 1.2 M ammonium chloride-ammonium hydroxide buffer (30 ml., pH 8.5), calcium chloride solution (22%, 2.0 ml.) and sodium taurocholate solution (25%, 0.1 ml.). A preparation of pork pancreatic lipase (100 mg., purified by homogenising with acetone, centrifuging and drying in a vacuum desiccator) was added and the pH was held at 8.5 by continual addition of ammonia (s.g. 0.880) whilst hydrolysis proceeded for 10 minutes. The solution was then adjusted to pH 1 with 2 N hydrochloric acid and extracted with ether (6 x 30 ml.).

The free fatty acids were removed by passing through a column of Amberlite IRA400 resin (30 g.), previously treated with sodium hydroxide and the neutral glycerides were recovered by evaporating the ether under reduced pressure. These were then chromatographed on a column of silica gel (Whatman chromedia, SG31) into triglycerides (eluted with benzene), diglycerides (eluted with benzene-ether 9:1) and monoglycerides (eluted with ether, for details see page 60 , Part I). The monoglycerides were examined by gas liquid chromatography on 20% polyetheleneglycol succinate column (see page 64, Part I).

Isolation of Lipolysis Products by T.L.C.

Thin layer plates (20 x 20 cm.) were prepared according to Stahl[43] (see page 59). The plates after air drying, were activated at 110° for 1 hour and cooled to room temperature. The total lipolysis product (10-20 mg., 10% in petrol solution) was applied as a band with a microsyringe (50 µl) on a line about 2 cm. from the bottom edge of the plate. The plates were developed (40 min.) by ascending elution with a mixture of acetone and chloroform (80 ml.:20 ml.) to which ammonia was added (1.5 ml., s.g. 0.880). After development the plates were dried in a current of nitrogen, sprayed with an ethanolic solution (0.2%) of dichlorofluorescein and separated components then appeared as bright yellow fluorescent zones when viewed under ultra violet light. The mixture separated into four well defined zones in the following order: triglycerides, diglycerides, monoglycerides and the acids (as their NH_4 salts) which remained at the base line.

The monoglycerides zone was placed in certrifuge tubes containing (6 x 10 ml.) ether and after stirring (1 min.), centrifuged and decanted. The contents were evaporated and analysed by gas liquid chromatography (see page 64) after transesterification with sodium in methanol.

Preparative Gas Liquid Chromatography on an Autoprep (Aerograph)

The Macadamia ternifolia and Gevuina avellana seed oils esters were subjected to preparative chromatography on a Apiezon L. (10%) column at 220°C, utilizing a hot wire detector. The fractions of C16, C18, and higher esters were collected by passing the effluent gas through collecting tubes packed with glass wool..

The purity of each fraction was checked by resampling on the Perkin Elmer Fractometer.

Fraction	Identification
1	Hexadecanoic and hexadecenoic
2	Octadecanoic, octadecenoic and octadecadienoic
3	Arachidic, behenic and eicosenoic

Separation of the Methyl Esters (Fraction 2) of G. avellana by Silver Nitrate Silicic Acid Chromatography[44,45]

A mixture of the silver nitrate-silica adsorbent (30 gm., see page 62) and light petrol ether was heated to boiling for five minutes while stirring. After cooling to room temperature, the slurry was brought into the column shielded from light.

A solution of methyl esters (187 mg.) in light petrol ether (5 ml.) was added to the column.

Fn	Eluant P. ether:ether	Volume (ml.)	Weight (mg.)	Identity
1	100 : 0	50	18	saturated + traces of monoene
2	95 : 5	50	46	monoene
3	95 : 8	50	83	
4	90 : 10	50	10	monoene + traces of diene
5	E	100	25	diene
	Total		182	

The purity of each fraction was again checked by gas liquid chromatography.

Von Rudloff oxidation of the Unsaturated Acids[20]

The esters (40-50 mg.) were dissolved in a solution of potassium carbonate (40 mg.) in water (16 ml.); butanol (48 ml.) and solution of potassium permanganate (5 mg.) and potassium periodate (280 mg.) in water (16 ml.) were added. The mixture was shaken for 24 hours at room temperature and excess oxident destroyed with sulphur dioxide. The solution was neutralised with potassium carbonate and reduced in volume on a rotary film evaporator. The acidified solution was saturated with sodium chloride and extracted thoroughly with ether (5 x 20 ml.). Evaporation of the ether afforded acids which were esterified with dry methanolic hydrogen chloride (see page 64) and examined by gas liquid chromatography on both Apiezon L. (10%) and polyethylene glycol succinate columns (20%) at appropriate temperatures.

Oil	Esters	Degradation products		Structure
G. avellana	Hexadec-enoic	monobasic C5	dibasic C11	Hexadec-11-enoic acid
	Octadec-enoic	C9	C9	Octadec-9-enoic acid
M. ternifolia	Hexadec-enoic	C7	C9	Hexadec-9-enoic acid

Calculations and Results

The fatty acid compositions of the whole triglycerides, and the resulting monoglycerides, for the seed oils we examined, are given in Table 12.

From the difference between these two sets of values the composition of the fatty acids occupying the $C_{(1)}$ and $C_{(3)}$ positions of the triglycerides can be calculated thus:-

	0	1	2	
Composition of triglycerides (Sunflower Nigerian)	10.1	30.4	59.5	a
Composition of triglycerides x 3	30.3	91.2	178.5	a x 3
Composition of monoglycerides	1.4	25.0	73.6	b
	28.9	66.2	104.9	
Composition of 1:3 acids	14.4	33.1	52.5	$\frac{(a \times 3-b)}{2}$

The triglyceride composition can then be calculated by distributing the 1:3 acids at random between the unoccupied positions of the monoglycerides, thus:-

Monoglycerides	% mol.	Diglycerides
		(00) $1.4 \times \frac{14.4}{100}$
0 (saturated)	1.4	(10) $1.4 \times \frac{33.1}{100}$
		(20) $1.4 \times \frac{52.5}{100}$

% mol.	Triglycerides	% mol.
	(000) $0.20 \times \frac{14.4}{100}$	0.03
0.20	(001) $0.2 \times \frac{33.1}{100}$	0.07
	(002) $0.2 \times \frac{52.5}{100}$	0.11
	(010) $0.46 \times \frac{14.4}{100}$	0.07
0.46	(011) $0.46 \times \frac{33.1}{100}$	0.15
	(012) $0.46 \times \frac{52.5}{100}$	0.24
	(020) $0.74 \times \frac{14.4}{100}$	0.11
0.74	(021) $0.74 \times \frac{33.1}{100}$	0.24
	(022) $0.74 \times \frac{52.5}{100}$	0.39

The calculation is extended to the monoglycerides containing oleic (1) and linoleic (2) acids to complete the analysis and then isomeric glycerides are added together. The results obtained in this way are given in Tables under columns "L".

Glyceride composition of seed oils can be calculated directly from the component acids with the assumption that the $C_{(2)}$ hydroxyl group is preferentially acylated by unsaturated C18 acids and the $C_{(1)}$ and $C_{(3)}$ hydroxyl groups are acylated susequently by all remaining acids and by any C18 unsaturated acid not required at $C_{(2)}$ thus:-

	0	1	2
Composition of triglycerides	10.1	30.4	59.5
Composition of Calc. 2 monoglycerides	-	33.9	66.1

$$\text{where oleic (1)} = \frac{\text{oleic}}{\text{oleic + linoleic}} \times 100$$

$$\text{linoleic (2)} = \frac{\text{linoleic}}{\text{oleic + linoleic}} \times 100$$

From the 2-monoglycerides (calc.) and the triglycerides the component glycerides can be derived similarly as described above.

TABLE 12

Component Esters (% mol.) of Triglycerides and 2-Monoglycerides

	14:0	16:0	18:0	16:1	18:1	18:2	18:3	Unsat.
1. Safflower (extract 30%, triglycerides 91%)								
triglycerides	-	6.6	3.4	0.6	12.2	77.0	0.2	90.00
2-monoglycerides	-	1.0	0.1	0.1	12.5	86.3	-	98.9
enrichment factor	-	0.01	-	-	1.02	1.12	-	1.10
selectivity factor	-	-	-	-	0.92	1.02		
2. Tobacco (supplied as crude oil, triglycerides 89%)								
triglycerides	-	9.8	3.8	1.0	13.5	70.6	1.3	85.4
2-monoglycerides	-	0.9	0.3	0.2	15.8	82.2	0.6	98.8
enrichment factor	-	0.09	0.07	0.02	1.17	1.17	0.46	1.16
selectivity factor	-	-	-	1.01	1.01	-		
3. J. gossypifolia (extract 27%, triglycerides 87%)								
triglycerides	0.3	7.7	5.8	0.4	16.5	69.3	-	86.2
2-monoglycerides	0.3	1.0	0.2	0.2	18.0	80.3	-	98.5
enrichment factor	1.0	0.01	0.03	0.5	1.08	1.16		1.14
selectivity factor	-	-	-	-	0.94	1.02		
4. Sunflower (Nigerian, extract 32%, triglycerides 99%)								
triglycerides	0.2	7.1	2.8	0.4	30.0	59.5	-	89.9
2-monoglycerides	-	1.1	0.3	-	25.0	73.6	-	98.6
enrichment factor	-	0.15	0.11	-	0.83	1.24	-	1.10
selectivity factor	-	-	-	-	0.75	1.12	-	
5. Sunflower (Bulgarian, extract 31%, triglycerides 99%)								
triglycerides	-	7.0	4.4	0.3	26.0	62.3	-	88.6
2-moroglycerides	-	1.1	0.3	-	23.9	74.7	-	98.6
enrichment factor	-	0.16	0.07	-	0.92	1.12	-	1.11
selectivity factor	-	-	-	-	0.83	1.01	-	-

TABLE 12 Contd.

	14:0	16:0	18:0	16:1	18:1	18:2	18:3	Unsat.
6. Sunflower (Nigerian, extract 33%, triglycerides 99%)								
triglycerides	0.2	5.1	1.8	0.5	50.3	42.1	-	92.9
2-monoglycerides	-	1.0	0.2	-	49.9	48.9	-	98.8
enrichment factor	-	0.19	0.11	-	0.99	1.16	-	1.06
selectivity factor	-	-	-	-	0.93	1.09	-	-
7. J. multifida (extract 40%, triglycerides 79%)								
triglycerides	-	19.1	7.1	1.3	23.3	49.2	-	73.80
2-monoglycerides	-	2.6	0.9	0.6	23.7	72.2	-	96.5
enrichment factor	-	0.14	0.01	0.46	1.02	1.47	-	1.31
selectivity factor	-	-	-	-	0.78	1.12	-	-
8. A. mexicana (extract 39%, triglycerides 87%)								
triglycerides	-	12.3	4.2	0.3	28.1	55.1	-	83.5
2-monoglycerides	-	2.2	-	0.5	35.0	62.2	-	97.7
enrichment factor	-	0.19	-	1.67	1.24	1.13	-	1.17
selectivity factor	-	-	-	-	1.06	0.96	-	-
9. Maize (supplied as crude oil, triglycerides 93%)								
triglycerides	-	12.6	1.8	0.8	30.0	54.3	0.5	86.6
2-monoglycerides	-	2.4	-	0.3	29.1	68.2	-	97.6
enrichment factor	-	0.19	-	0.37	0.97	1.26	-	1.13
selectivity factor	-	-	-	-	0.86	1.12	-	-
10. Cotton (extract 20%, triglycerides 83%)								
triglycerides	1.1	27.3	3.1	1.4	16.7	50.4	-	68.5
2-monoglycerides	0.3	3.6	0.3	0.7	24.5	70.6	-	95.8
enrichment factor	0.27	0.13	0.09	0.5	1.47	1.40	-	1.40
selectivity factor	-	-	-	-	1.05	1.0	-	-

TABLE 12 Contd.

	14:0	16:0	18:0	16:1	18:1	18:2	18:3	Unsat.
11. Groundnut (extract 50%, triglycerides 91%)								
triglycerides[a]	-	9.8	3.7	0.4	60.9	18.1	-	79.4
2-monoglycerides	-	1.8	0.2	0.4	66.6	31.0	-	98.0
enrichment factor	-	0.18	0.54	0.1	1.09	1.7	-	1.23
selectivity factor	-	-	-	-	0.89	1.38	-	-
12. M. ternifolia (extract 74%, triglycerides 94%)								
triglycerides[b]	0.7	9.3	3.7	27.2	51.9	2.8	-	81.9
2-monoglycerides	-	0.9	-	23.8	71.0	4.3	-	99.1
enrichment factor	-	0.09	-	0.87	1.36	1.53	-	1.21
selectivity factor	-	-	-	0.72	1.13	1.26	-	-
13. M. ternifolia (extract 68%, triglycerides 93%)								
triglycerides[c]	0.5	10.1	6.2	18.3	55.4	3.4	-	77.1
2-monoglycerides	-	0.8	-	15.5	77.6	6.1	-	99.2
enrichment factor	-	0.06	-	0.85	1.40	1.76	-	1.28
selectivity factor	-	-	-	0.66	1.09	1.37	-	-
14. G. asiatica (extract 60%, triglycerides 96%)								
triglycerides[d]	-	10.1	8.1	0.3	28.8	37.6	-	69.4
2-monoglycerides	-	1.6	-	0.9	37.3	60.2	-	98.4
enrichment factor	-	0.15	-	0.3	1.29	1.65	-	1.42
selectivity factor	-	-	-	0.90	1.16	-	-	-
15. J. curcas (extract 30%, triglycerides 99%)								
triglycerides	-	15.9	4.3	2.9	39.7	37.2	-	79.8
2-monoglycerides	-	2.3	0.2	1.1	41.1	55.3	-	97.5
enrichment factor	-	0.14	-	0.38	1.03	1.49	-	1.22
selectivity factor	-	-	-	-	0.34	1.22	-	-

TABLE 12 Contd.

	14:0	16:0	18:0	16:1	18:1	18:2	18:3	Unsat.
16. *M. latifolia* (extract 46%, triglycerides 94%)								
triglycerides	-	23.7	24.1	0.2	37.8	14.4	-	52.2
2-monoglycerides	-	3.1	4.5	-	60.4	32.0	-	92.4
enrichment factor	-	0.13	0.19	-	1.61	2.22	-	1.77
selectivity factor	-	-	-	-	0.90	1.25	-	--

Seed oils containing hexadecenoic, eicosenoic and docosenoic acids

	16:0	18:0	20:0	22:0	16:1	18:1	18:2	18:3	20:1	20:2	22:1	Unsat.
17. *G. avellana* (extract 41%, triglycerides 91%)												
triglycerides	2.3	0.6	1.4	2.0	24.0	43.7	7.3	-	9.9	-	8.3	75.5
2-monoglycerides	0.5	-	-	-	26.6	57.3	15.6	-	-	-	-	99.5
enrichment factor	-	-	-	-	1.1	1.3	2.0	-	-	-	-	1.31
selectivity factor	-	-	-	-	0.8	1.0	1.52	-	-	-	-	-

	16:0	18:0	20:0	22:0	16:1	18:1	18:2	18:3	20:1	22:2	22:1	Unsat.
18. *G. avellana* (extract 39%, triglycerides 89%)												
triglycerides	2.2	0.7	1.5	1.9	23.9	41.8	8.8	-	10.3	8.9	-	74.5
2-monoglycerides	0.4	-	-	-	26.3	54.7	18.6	-	-	-	-	79.6
enrichment factor	-	-	-	-	1.1	1.3	2.1	-	-	-	-	1.07
selectivity factor	-	-	-	-	1.03	1.21	1.96	-	-	-	-	-

TABLE 12 Contd.

	16:0	18:0	20:0	22:0	10:1	18:1	18:2	18:3	20:1	22:2	22:1	Unsat.
19. C. halicacabum (extract , triglycerides 99%)												
triglycerides[e]	3.9	1.7	12.8	0.5	-	25.1	8.9	1.3	40.4	-	2.6	35.3
2-monoglycerides	4.1	0.8	3.5	-	-	49.8	20.3	-	16.7	-	-	70.1
enrichment factor	-	-	0.27	-	-	1.98	2.28	-	0.41	-	-	1.98
selectivity factor	-	-	-	-	-	1.0	1.15	-	-	-	-	-

Seed oils containing lauric and myristic acids

	12:0	14:0	16:0	18:0	20:0	12:1	14:1	16:1	18:1	18:2	20:1
20. I. gabonensis (extract 67%, triglycerides 92%)											
triglycerides	33.5	48.6	10.2	0.5	-	2.4	-	Tr	4.8	-	-
2-monoglycerides	14.6	76.0	8.9	-	-	-	-	0.5	-	-	-
enrichment factor	0.43	1.56	0.87	-	-	-	-	-	-	-	-
[illegible]	-	-	-	-	-	-	-	"	"	"	
21. P. angolensis (extract 71%, triglycerides 76%)											
triglycerides	11.6	74.9	1.5	-	-	-	11.0	1.0	-	-	-
2-monoglycerides	20.1	65.1	2.8	-	-	-	9.9	2.1	-	-	-
enrichment factors	1.73	0.87	-	-	-	-	0.9	-	-	-	-
22. M. malabarica (extract 40%, triglycerides 96%)											
triglycerides	-	39.9	13.5	2.6	0.8	-	3.3	3.4	28.3	6.0	2.2
2-monoglycerides	-	44.9	5.3	-	0.7	-	3.6	2.4	31.4	9.7	1.0
enrichment factor	-	1.12	0.39	-	-	-	-	0.71	1.11	1.12	0.45

TABLE 12 Contd.

	20:0	22:0	24:0	20:1	22:1	20:2
"a" also	1.4	2.7	1.4	1.3	0.3	-
"b" also	2.4	-	-	2.0	-	-
"c" also	3.7	-	-	2.4	-	-
"d" also	2.6	2.4	-	9.9	0.2	-
"e" also	-	-	-	-	-	2.8
	-	-	-	-	-	4.8

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Determination of the Component Glycerides of Seed Oils containing Saturated Oleic and Linoleic Acids

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Jatropha curcas seed oil[1] which contains palmitic acid (16·4%), hexadecenoic acid (1·3%), stearic acid (6·4%), oleic acid (39·8%), and linoleic acid (36·1%),* can be separated into nine zones on thin layers of silica gel (Merck) impregnated with silver nitrate[2] by development with benzene-ether (9:1). These have been shown to contain the following glycerides: trilinolein (L_3), dilinoleo-olein (L_2O), dilinoleo-saturated glycerides (L_2S), linoleo-diolein (LO_2), linoleo-oleo-saturated glycerides (LOS), triolein (O_3), linoleo-disaturated glycerides (LS_2), dioleo-saturated glycerides (O_2S), and oleo-disaturated glycerides (OS_2). This system does not separate isomeric glycerides such as the symmetrical and unsymmetrical linoleo-oleins but glycerides with the same total unsaturation, such as L_2S and LO_2, LOS and O_3, and LS_2 and O_2S, are well resolved. We have developed two quantitative procedures for determining component glycerides based on this thin-layer separation.

The oil (4–15 mg. in ether solution) is applied as a band to a glass plate (20 × 20 cm. or 20 × 40 cm.) layered with silica gel (275μ) containing 15% of silver nitrate and developed with benzene-ether (9:1) in a conventional tank or by horizontal elution.[3] Two narrow strips in the direction of development, each about one in. from the edge of the plate, are made visual by drawing the tip of a small flame from a glassblower's torch along the plate. The adsorbent between these two strips is scraped from the plate in bands which correspond to the developed spots and the adsorbed triglycerides are recovered by extraction twice with ether-methanol-water (1:1:0·2) and four times with ether-methanol (1:1). The extracts are too small to be weighed with convenience so a known amount of methyl heptadecanoate (0·4–1·5 mg.) is added to each extract before it is converted to methyl esters for quantitative examination by gas-liquid chromatography. (Perkin Elmer Fractometer, 1 m. column containing polyethylene glycol succinate as stationary phase, flame ionisation detector). From the peak areas for each ester and the C_{17} ester the composition of each fraction and its relative amount are determined. Each extract contains one major triglyceride accompanied by minor amounts of one or two others and its glyceride composition can be derived from the molar proportions of the component esters. The concentration of the major glyceride in each extract is usually between 85 and 95%. This technique of adding a known amount of a marker to extracts from a chromatogram could be used in other quantitative studies based on separations by thin-layer chromatography.

In the second technique the effective thin-layer separation has been used to monitor separations by other procedures. Low-temperature crystallisation of mixtures of glycerides from organic solvents gave a poor separation when linoleic-containing glycerides accompanied oleic-containing glycerides but crystallisation from mixtures of acetone and methanolic silver nitrate was more effective. The oil is separated into three fractions in which monounsaturated glycerides (US_2), diunsaturated glycerides (U_2S), and triunsaturated glycerides (U_3) are separately concentrated by crystallisation at −10 to −20°C. and at −70°C. The simpler mixtures so obtained, but not the original oil, can then be eluted from columns of silica impregnated with silver nitrate (25%) with benzene-ether mixtures in fractions, shown by thin-layer chromatography, to contain not more than two triglycerides. The composition of such fractions is derived from a knowledge of their component acids determined by gas liquid chromatography. This improved separation of glycerides in the presence of silver nitrate can be carried out on any scale and provides a new and effective separation procedure.

Table

	L_3	L_2O	L_2S	LO_2	LOS	O_3	LS_2	O_2S	OS_2
Method 1	5	14	12	15	19	10	7	13	5
Method 2	4	17	10	15	24	8	7	12	3
Calc.	4	15	10	17	22	7	6	13	6

These two procedures were applied to *J. curcas* seed oil and the final results are given in the table. They compare favourably with each other and with values calculated according to a theory of acyl group distribution proposed by one of us.[4]

We hope to link these methods with enzymic hydrolysis to get further information about the structure of these glycerides and we are also applying these methods to oils containing more highly unsaturated acids.

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* All values of percentage composition are quoted on a molar basis. Hexadecenoic acid is hereafter included with oleic acid.

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47. *Glyceride Studies. Part II.*[1] *The Component Glycerides of Seed Oils Containing Saturated, Oleic, and Linoleic Acids.*

By F. D. Gunstone, R. J. Hamilton, and M. Ilyas Qureshi.

The component glycerides of three *Jatropha* oils, containing only palmitic, stearic, oleic, and linoleic acid, have been determined by lipolysis and by a new method of crystallisation and column chromatography. The results differ from those previously obtained for seed oils of similar composition, but the two methods give values which agree with one another and with those calculated according to a theory previously postulated. Certain refinements of this theory are proposed. Low-temperature crystallisation of glycerides in presence of silver nitrate gives an improved separation of these compounds according to their degree of unsaturation.

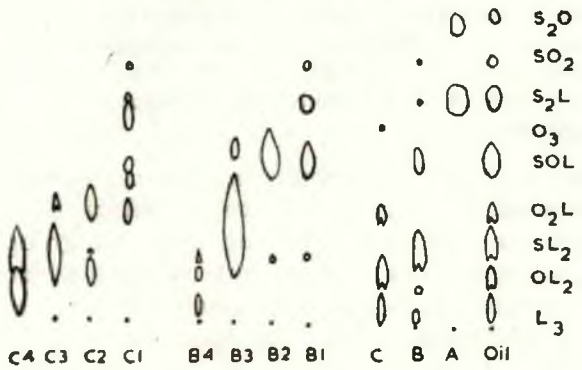
In an earlier Paper [1] three theories concerning the distribution of acyl groups in natural triglycerides were examined and it was concluded that the results then available for seed oils were consistent with the view that the secondary glycerol hydroxyl group is preferentially acylated with unsaturated C_{18} acids and that the two primary hydroxyl groups are acylated subsequently with saturated acids and with unsaturated C_{18} acids not required at the 2-position. Mattson and Volpenhein [2] had previously shown that, in seed oils, monoethenoid C_{20} and C_{22} acids do not compete with the C_{18} unsaturated acids for the 2-position but are present almost entirely at positions 1 and 3. This theory of acyl-group distribution is accepted by Vander Wal [3] and by Coleman,[4] but it lacks experimental proof. We have now examined three *Jatropha* oils by two methods; the results confirm and extend the above theory of acyl-group distribution.

Methods.—Each oil has been hydrolysed with pork pancreatic lipase which preferentially removes acyl groups from positions 1 and 3, leaving the 2-monoglycerides. The 2-monoglycerides were isolated by adsorption chromatography and their component acids determined, as methyl esters, by gas–liquid chromatography. From the composition of the triglycerides and of the 2-monoglycerides derived from them it is possible to calculate glyceride composition if it is *assumed*, as Coleman [4] and Vander Wal [3] do, that the possible combinations of 1- and 3-acyl groups with those at C-2 are statistically arranged. Values are obtained for every possible triglyceride, including isomers, but these have been grouped together into the main categories shown in Table 5. The value shown against SOL, for example, is the total value for the six triglycerides containing one linoleic acid group, one oleic acid group, and one palmitic or stearic acid group.

A second method of glyceride analysis has been devised which gives information about component glycerides without any such assumption. Thin-layer chromatography on silica impregnated with silver nitrate gives very effective separation of triglycerides according to their unsaturation (see Figure).[5] The *Jatropha* oils show nine spots, corresponding to trilinolein (L_3), oleodilinolein (OL_2), saturated dilinoleins (SL_2), dioleolinolein (O_2L), saturated oleolinoleins (SOL), triolein (O_3), disaturated linoleins (S_2L), saturated dioleins (SO_2), and disaturated oleins (S_2O), and we have used this technique to monitor the efficacy of separations by other methods. Low-temperature crystallisation has been much used in the past for glyceride studies,[6] but some of the results are now suspect [1,7] and we have confirmed the incompleteness of these separations by thin-layer chromatography. In the presence of silver nitrate, however, we obtained much better separations. The neutralised seed oils, chromatographed on silica [8] to remove partial glycerides when necessary, are crystallised from dilute solutions in acetone and methanolic silver nitrate.

At −10° to −20° the precipitate (fraction A) is mainly disaturated monounsaturated glycerides (S_2U) with some monosaturated glycerides (SU_2) [trisaturated glycerides (S_3) are absent from these oils]; cooling to −70° gives more crystals (fraction B), mainly SU_2 with a little U_3; the mother-liquor is almost entirely U_3. This extension of the use of silver nitrate in chromatography and counter-current distribution [9] can be carried out on any scale and provides a useful separation technique. A single crystallisation at −70°, for example, readily affords, in high yield, a fraction which is almost entirely triunsaturated glycerides.

Attempts to isolate binary or ternary mixtures of glycerides by column chromatography on silica impregnated with silver nitrate failed with the seed oils but were successful with the separated fractions A, B, and C; this was demonstrated by thin-layer chromatography (see Figure). By these means the oil is divided into 9—11 fractions, and the component



Thin-layer chromatogram of *Jatropha multifida* seed oil and glyceride fractions.

acids of each are determined, after transesterification, by gas–liquid chromatography. The sum of the acid increments from the analysed fractions shows a small loss of linoleic acid and we have adjusted the linoleic acid figures upwards, and all other values correspondingly downwards, to correct for this. Similar losses, often more serious, have commonly been encountered in glyceride studies.[10] The composition of each fraction, after adjustment, is then converted from a weight-percentage basis into a molar-percentage basis. Qualitative evidence of the glycerides present, from the thin-layer chromatograms, is combined with the quantitative information about the component acids and in almost all cases the amounts of component glycerides can be calculated. Fractions which cannot be so handled are usually small and reasonable assumptions can be made without introducing serious error into the final results (see Tables 2—5).

Results.—The *Jatropha* genus belongs to the family Euphorbiaceæ and information about *Jatropha* seed oils, previously limited to the component acids of *J. curcas*,[11] *J. glandulifera*,[12] and *J. macrocarpa*,[13] is now extended to two further species, *J. multifida* and *J. gossypifolia*. The results are summarised in the annexed Table; the *J. gossypifolia* seed oil with its high content of linoleic acid is of particular interest.

Jatropha oils.

Component acids (wt.-%)	*J. curcas*	*J. glandulifera*	*J. multifida*	*J. macrocarpa*	*J. gossypifolia*
16 : 0 + 18 : 0 *	21·9	22·8	24·6	15·2	12·8
16 : 1 + 18 : 1	41·5	34·2	25·0	33·4	16·6
18 : 2	36·6	43·0	50·4	51·4	70·6

* The symbols used here and in the Tables indicate the number of carbon atoms and double bonds in each acid molecule. Thus 18 : 1 and 18 : 2 are used for oleic acid and linoleic acid, respectively.

Before discussing the consequences of our results for any theory of acyl-group distribution we note that they differ from those of seed oils of similar composition previously examined by the normal low-temperature crystallisation. The major glycerides for *J. multifida* seed oil are compared in our next Table with those of *Chorozophora plicata* [14]

and cottonseed oil [15] and those for *J. gossypifolia* are compared with results for tobacco-seed oil and sunflower-seed oil.

	C. plicata [14]	Cotton-seed [15]	*J. multifida*	Sunflower seed [17]	Tobacco seed [16]	*J. gossypifolia*
			Component acids (mol.-%)			
Saturated (S) ...	23	29	26	15	11	14
Oleic (O)	25	24	25	18	17	17
Linoleic (L) ...	52	47	49	67	72	69
			Component glycerides (mol.-%)			
SOL	28	41	22	6	3	8
SL_2	19	18	23	39	23	26
O_2L	—	—	9	—	—	6
OL_2	37	28	15	47	48	22
L_3	3	—	10	8	23	33

The agreement between the results obtained by crystallisation and chromatography, by lipolysis, and by calculation according to our earlier theory [1] (Table 5), indicates the general correctness of this theory and provides, for the first time, justification for the assumptions underlying the calculation of component glycerides from lipolysis results. A more detailed examination of these results, however, shows that the theory requires refinement in two ways. Our lipolysis results and the larger number of results obtained by others show that the unsaturated C_{18} acids are *not equally distributed at position* 2 but that, in most cases, there is a stronger preference for linoleic acid in this position than for oleic or linolenic acid.

This general phenomenon can be discussed in terms of an " enrichment factor " which we define as the ratio of the molar concentration of an acid in the 2-monoglyceride resulting from lipolysis to its molar concentration in the triglyceride. The enrichment factor can have any value between 0 and 3; values <1 indicate a preference for positions 1 and 3, values >1 for position 2. The results given in Table 6 are typical of a much larger range of values (not reproduced here) calculated from the results of seed oils examined by Mattson and Volpenhein [2,18] and others.[4,5,19] Enrichment factors are $<0{\cdot}2$ for palmitic, stearic, eicosenoic, and docosenoic acid; amongst the C_{18} unsaturated acids, linoleic consistently has a higher value than has oleic or linolenic acid.

Minor differences in the distribution of oleic and linoleic acid are also apparent from our crystallisation and chromatography results. When the oleic : linoleic ratios in fractions A (mainly S_2U), B(mainly SU_2), and C (mainly U_3) are compared with those in the oils they are consistently higher in fractions A and C and lower in fraction B (Table 6).

In our results there is also some evidence, which we intend to examine further, that the saturated acids, palmitic and stearic, show minor differences in their behaviour. Comparison of the palmitic : stearic ratios in fractions A, B, and C, and in some of the chromatographic fractions, with those for the oils shows an enhanced ratio in the monosaturated glycerides (SU_2) and a depressed one in the disaturated glycerides (S_2U).

There remains, therefore, the following scheme of acyl group distribution in natural triglycerides of vegetable origin. Unsaturated C_{18} acids are esterified at position 2, with linoleic acid taking slight precedence over oleic and linolenic acid; there is a tendency for palmitic acid and linoleic acid to be enriched in monosaturated glycerides (SU_2), for stearic acid and oleic acid to be enriched in disaturated glycerides (S_2U), and for oleic acid to be enriched in triunsaturated glycerides (U_3).

Experimental

Whenever possible operations were carried out under nitrogen. Glycerides, esters, and acids were stored under nitrogen at 0°. Light petroleum is the fraction of boiling range 40—60° unless otherwise designated.

Gas–Liquid Chromatography.—Quantitative gas–liquid chromatography was carried out with a Perkin-Elmer Fractometer and a 1-m. column of firebrick (60—80 mesh) coated with poly(ethylene glycol succinate) (20%). This was operated at 190° with a flame ionisation

detector, and peak areas were obtained by multiplying the peak height by the width at half-height or, in some cases, by planimetry. Results are the mean of at least two chromatograms.

Thin-layer Chromatography.—To separate mono-, di-, and tri-glycerides a 2% light petroleum solution (1 μl.) was applied to glass plates covered with a layer of Merck's silica gel G (270 μ thick). These were developed with benzene–ether (17 : 3) and spots became visible when the plate was sprayed with 50% sulphuric acid and charred at 200°.

Glycerides of varying unsaturation were separated on layers of silica gel impregnated with silver nitrate (17%). The glycerides were applied in 1% light petroleum solution (0·5—1·0 μl.) and the plates were developed with benzene–ether (9 : 1) for about 40 min. After the solvent had evaporated from the plate the glycerides were made visible as dark charred spots by drawing a small hot flame from a glassblower's torch across the plate.

Transesterification.—Triglycerides were converted into methyl esters by reaction with anhydrous methanolic hydrogen chloride [20] or by reaction with sodium in dry methanol.[21]

Preparation of Neutral Triglycerides.—Samples of *J. curcas* seeds (Ibadan), *J. multifida* seeds (Achimoto), and *J. gossypifolia* seeds (Achimoto) were crushed and extracted with light petroleum in a Soxhlet extractor. The extracted oils, dissolved in chloroform, were percolated through a column of alumina (Peter Spence, Type H, 100—200 mesh) to remove free fatty acids. When thin-layer chromatography showed the presence of mono- and di-glycerides in the neutralised oil, a chloroform solution (1 g. in 15 ml.) was percolated through a column (33 × 1·8 cm.) of silica gel (30 g.).[8] Triglycerides (benzene), diglycerides (benzene–ether, 9 : 1), and monoglycerides (ether) are eluted in that order by the solvents shown. The complete removal of partial glycerides from triglycerides and the complete recovery of the latter were checked by thin-layer chromatography.

J. curcas kernels contain 49% of oil (30% based on seeds) of which 99% was recovered after neutralisation with alumina. The *J. multifida* kernels contain 52% of oil (40% based on seeds); 99% of this was recovered as neutral oil and separated into triglycerides (80%), diglycerides (19%) and monoglycerides (1%). The *J. gossypifolia* oil (27% from kernels) gave 95% of neutral oil separated into triglycerides 92%, diglycerides 6%, and monoglycerides 2%.

Lipolysis.[4]—The triglycerides (1·30 g.) were stirred in a double-walled vessel, kept at 37° by circulating water between the two walls, along with a 1·2M-ammonium chloride–aqueous ammonia buffer (30 ml.; pH 8·5), 22% calcium chloride solution (2·0 ml.), and 25% sodium taurocholate solution (0·1 ml.). A preparation of pork pancreatic lipase (100 mg., purified by homogenising it with acetone, centrifuging, and drying in a vacuum-desiccator) was added and the pH was held at 8·5 by continual addition of aqueous ammonia (*d* 0·880) whilst hydrolysis proceeded for 10 min. The solution was then adjusted to pH 1 with 2N-hydrochloric acid and extracted with ether (6 × 30 ml.). The ethereal solution was passed through a column of Amberlite IRA-400 resin (30 g.), previously treated with sodium hydroxide; the eluted glycerides (0·93 g.), chromatographed on silica gel (Davidson Grade 923, 100—200 mesh), gave triglycerides (0·20 g., eluted with benzene), diglycerides (0·33 g., eluted with benzene–ether, 9 : 1), and monoglycerides (0·31 g., eluted with ether). The last fraction was examined by gas–liquid chromatography after transesterification.

Low-temperature Crystallisation.—A solution of *Jatropha* triglycerides (1·5—2·0 g.) in a saturated solution of methanolic silver nitrate (~2·5%; containing twice the amount of silver nitrate required to form complexes with all the olefinic centres) and acetone (3 ml. per 7 ml. of methanol) was kept at −10° for 24 hr., then quickly filtered through a sintered-glass filter cooled to −15°. The precipitate (fraction A) was twice washed with a little cold acetone–methanolic silver nitrate. The filtrate was held at −70° for 24 hr. and the precipitate (fraction B) was filtered from the mother-liquor (fraction C). The precipitated glycerides were dissolved in light petroleum (25 ml.) and washed with distilled water (3 × 50 ml.) to remove silver nitrate, and the aqueous washings were re-extracted with light petroleum (40 ml.). Solvent was removed from the petroleum solution after drying. The mother-liquor was concentrated to 20 ml., washed into a separatory funnel with petroleum (50 ml.), and treated as above.

Column Chromatography.[5]—Silver nitrate was deposited on silica gel (Whatman, Silica Gel, SG 31) by suspending the latter (100 g.) in water (100 ml.) containing silver nitrate (33 g.). The resulting slurry was heated in an oven at 100° until most of the water had evaporated and then at 120° for 16 hr. The cold dry adsorbent was finally passed through a 60-mesh sieve. A slurry of this adsorbent (30 g.) in dry benzene (150 ml.) made a column 32 × 1·6 cm. in a tube protected from light with black paper. A solution of each of the fractions A, B, and C

(100—150 mg.) in benzene (5 ml.) was placed on the column and eluted with 200 ml. each of a range of solvents of increasing polarity. Each eluate was washed with distilled water (3 × 40 ml.) to remove silver nitrate, dried, and weighed after removal of solvent. Four to eight fractions were usually collected; these were subsequently examined by thin-layer chromatography on a silicic acid–silver nitrate plate, and fractions of similar composition combined.

Results.—Calculations are collected in the Tables.

TABLE 1.

Component acids (mol.-%)	14 : 0	16 : 0	18 : 0	16 : 1	18 : 1	18 : 2
J. curcas						
Triglyceride	—	15·9	4·3	2·9	39·7	37·2
2-Monoglyceride	—	2·3	0·2	1·1	41·1	55·3
J. multifida						
Triglyceride	—	19·1	7·1	1·3	23·3	49·2
2-Monoglyceride	—	2·6	0·9	0·6	23·7	72·2
J. gossypifolia						
Triglyceride	0·3	7·7	5·8	0·4	16·5	69·3
2-Monoglyceride	0·3	1·0	0·2	0·2	18·0	80·3

TABLE 2.

Jatropha curcas oil.

Component acids (wt.-%) of seed oil, fractions from low-temperature crystallisation, and fractions from column chromatography

	Wt.-%	14 : 0	16 : 0	18 : 0	16 : 1	18 : 1	18 : 2
J. curcas oil		Tr	15·4	6·5	1·3	40·2	36·6
Fraction A	20·4	1·8	32·6	17·7	1·4	28·3	18·2
B	37·1	0·1	24·1	8·8	2·0	30·1	34·9
C	42·5	—	0·5	—	0·9	54·0	44·6
A1 (PB50) *	3·8	0·4	39·8	20·3	2·3	32·0	5·2
A2 (PB70)	4·2	—	42·0	18·9	0·8	17·9	20·4
A3 (B)	9·0	—	27·9	17·0	1·5	34·6	19·0
A4 (E)	3·4	0·2	21·0	15·3	1·2	31·0	31·3
B1 (B)	17·2	0·2	22·4	8·4	2·2	46·2	20·6
B2 (BE5)	15·7	0·3	24·1	8·0	1·8	18·4	47·4
B3 (E)	4·2	—	20·0	7·0	1·7	14·6	56·7
C1 (BE2)	13·9	0·2	1·6	—	1·7	83·2	13·3
C2 (BE2)	10·8	0·1	1·0	—	2·0	59·3	37·6
C3 (BE5)	5·1	—	2·3	—	2·0	41·0	54·7
C4 (E)	12·7	0·1	0·9	—	1·4	26·4	71·2
[Total		0·1	15·5	6·6	1·8	41·0	35·0]

Iodine value of triglycerides = 97·8 (calc. from above results 99·4).

* Eluting solvents: P = light petroleum (b. p. 40—60°), B = benzene, E = ether; a number indicates the amount (v/v %) of the second solvent in the mixture.

TABLE 3.

Jatropha multifida oil.

Component acids (wt.-%) of seed oil, fractions from low-temperature crystallisation, and fractions from column chromatography

	Wt.-%	16 : 0	18 : 0	16 : 1	18 : 1	18 : 2
J. multifida oil		16·9	7·7	1·5	23·5	50·4
Fraction A †	12·9	38·4	21·5	1·8	12·9	25·4
B	52·7	22·5	8·7	1·1	20·5	47·2
C	34·4	1·6	—	1·5	32·2	64·7
B1 (B) *	18·7	26·4	11·4	2·2	37·1	22·9
B2 (B)	6·9	17·7	10·3	2·0	35·1	34·9
B3 (BE5)	23·2	24·8	9·3	1·6	4·8	59·5
B4 (E)	3·9	8·0	3·3	1·2	13·2	74·3
C1 (BE2)	6·7	7·3	—	2·5	64·4	25·8
C2 (BE5)	8·0	3·2	—	2·6	51·2	43·0
C3 (BE10)	4·1	1·0	—	1·8	35·3	61·9
C4 (E)	15·6	1·6	—	1·4	15·0	82·0
[Total		18·2	7·9	1·9	24·8	47·2]

Iodine value of triglycerides = 107·3 (calc. from above results 109·3).

* See footnote to Table 2. † Fraction A was not further separated by column chromatography.

TABLE 4.

Jatropha gossypifolia oil.

Component acids (wt.-%) of seed oil, fractions from low-temperature crystallisation, and fractions from column chromatography

	Wt.-%	14 : 0	16 : 0	18 : 0	16 : 1	18 : 1	18 : 2
J. gossypifolia oil		0·6	7·0	5·2	0·4	16·2	70·6
Fraction A †	2·8	0·4	23·5	36·3	0·7	9·7	29·4
B	45·9	0·3	13·4	9·8	0·6	12·3	63·6
C	51·3	0·1	0·4	—	0·3	19·8	79·4
B1 (BP30) *	1·5	3·8	42·5	10·2	3·1	24·2	16·2
B2 (B)	2·2	1·4	24·2	11·0	0·3	41·8	21·3
B3 (B)	5·3	0·5	19·4	10·1	0·4	35·4	34·2
B4 (BE5)	23·8	0·4	18·3	11·8	0·2	6·9	62·4
B5 (BE5)	4·5	0·3	10·9	4·0	0·1	17·8	66·9
B6 (E)	8·6	0·2	1·5	0·4	0·5	2·2	95·2
C1 (BE2)	5·2	1·0	7·8	1·5	2·5	57·9	29·3
C2 (BE5)	6·5	—	2·8	0·9	0·8	44·8	50·7
C3 (BE10)	26·6	—	0·4	—	0·6	17·5	81·5
C4 (E)	13·0	—	0·8	—	0·4	2·7	96·1
[Total		0·3	8·6	5·1	0·6	17·0	68·4]

Iodine value of triglycerides = 133·8 (calc. from above results 137·1).

* See footnote to Table 2. † See footnote to Table 3.

TABLE 5.

Component glycerides (mol.-%) of *Jatropha* oils.

	J. curcas			*J. multifida*			*J. gossypifolia*		
	A	B	C	A	B	C	A	B	C
S_2U	10	10	10	15	15	15	3	5	4
SU_2	47	41	44	50	47	48	35	32	33
U_3	43	49	46	35	38	37	62	63	63
S_3	—	0·2	—	—	0·5	—	—	0·1	—
S_2O †	3·5	4·2	5·6	4·2	4·1	5·0	1·1	0·8	0·8
S_2L	6·9	5·1	4·9	10·8	11·1	10·1	2·2	3·6	3·4
SO_2	11·6	11·0	12·4	4·6	4·7	5·2	1·1	1·2	1·2
SOL	23·8	21·2	21·8	23·1	21·0	21·1	7·6	10·1	10·1
SL_2	11·4	9·2	9·6	22·7	20·9	21·2	25·9	21·1	21·2
O_3	8·6	7·7	6·9	1·0	1·5	1·4	1·2	0·5	0·5
O_2L	14·7	20·3	18·1	9·1	9·0	8·3	6·1	5·9	5·7
OL_2	16·2	16·7	16·0	15·0	16·9	16·6	21·7	24·0	23·9
L_3	3·3	4·4	4·7	9·5	10·3	11·1	33·1	32·7	33·2

* A, Crystallisation and chromatography (M. I. Q.). B, Lipolysis (R. J. H.). C, Calc. according to theory 1.[1] † O includes 16 : 1 and 18 : 1.

TABLE 6.

Palmitic : stearic and oleic : linoleic ratios (%.-wt.).

	J. curcas		*J. multifida*		*J. gossypifolia*	
	P : St	O : L	P : St	O : L	P : St	O : L
Oil	2·37	1·10	2·20	0·47	1·35	0·23
Fraction A	1·84	1·56	1·79	0·51	0·65	0·33
B	2·74	0·86	2·58	0·43	1·37	0·19
C	—	1·21	—	0·50	—	0·25

Enrichment factors (%.-mol.)

	16 : 0	18 : 0	16 : 1	18 : 1	18 : 2
J. curcas	0·14	0·05	0·38	1·04	1·49
J. multifida	0·14	0·13	0·46	1·02	1·47
J. gossypifolia	0·13	0·03	0·50	1·09	1·16

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Glyceride Studies. Part IV. The Component Glycerides of Ten Seed Oils Containing Linoleic Acid

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Abstract

The component glycerides of ten seed oils (safflower, tobacco, sunflower, *Argemone mexicana*, maize, cotton, groundnut, *Macadamia ternifolia*, *Gmelina asiatica*, and *Madhuca latifolia*) have been estimated by chromatographic procedures. The results agree with those obtained by lipolysis or calculated directly from the component acids on the basis of the theory of positional distribution.

Introduction

OLEIC AND LINOLEIC are the only unsaturated acids present in many seed oils, including some that are available in large quantities, but information about their component glycerides, apart from a few recent analyses, is based largely on the low temperature crystallisation procedure now considered to be inadequate for the more unsaturated seed oils. We have examined ten seed oils by lipolysis and by chromatographic separation on silica impregnated with silver nitrate. It is convenient to divide these oils into four groups:

(i) Oils with a very high proportion of linoleic acid ($> 70\%$): safflower and tobacco.
(ii) Oils with a high proportion of linoleic acid (50–60%): sunflower, *Argemone mexicana*, maize, and cottonseed.
(iii) Oils with a high proportion of oleic acid ($> 50\%$): groundnut and *Macadamia ternifolia*.
(iv) Oils with a high content of "saturated" acids ($> 30\%$): *Madhuca latifolia* (Mowrah butter) and *Gmelina asiatica*.

Some of the oils fall into more than one category but we have chosen to include them in the group given above rather than in another.

Procedure

Seeds or extracted oils were obtained from J. Bibby and Sons (maize, cottonseed, and groundnut); from the Tropical Products Institute (safflower (var. U.S.A./P2) from Kenya, sunflower from Nigeria (var. Jupiter) and from Bulgaria, *Argemone mexicana* from Jamaica, *Macadamia ternifolia* from Tanganyika, *Madhuca latifolia* from Bombay, and *Gmelina asiatica* from Singapore); from Younghusband, Stephens, and Co. Ltd. (tobacco); and from Dr. C. Y. Hopkins (*M. ternifolia*).

Crushed seeds were thoroughly extracted with boiling petrol ether (bp 40–60C). The extracted oil was neutralised by percolation in chloroform solution through a column of alumina and the triglycerides were subsequently eluted from a column of silica (Whatman chromedia, SG31) with benzene; more polar solvents subsequently removed diglycerides and monoglycerides (1).

Lipolyses were carried out as described in our earlier papers (2,3). The two sunflower oils were examined by low-temperature crystallisation from acetone and methanolic silver nitrate followed by chromatography on columns of silica-silver nitrate (2). The remaining oils were examined by our thin-layer procedure (3,4), developing the plate (20×20 cm) with benzene containing 10% of ether. We find the latter procedure to be quicker and more satisfactory. The results are summarised in Tables I and III to V.

TABLE I

Component Esters (% mol) of the Whole Oil, the 2-Monoglycerides, and the Sum of the Separated Fractions

	14:0	16:0	18:0	16:1	18:1	18:2	18:3[a]
Safflower (extract 30%, triglycerides 91%, iodine value 141)							
Triglyceride	....	6.6	3.4	0.6	12.2	77.0	0.2
Plate (9 fractions)	....	7.0	2.8	0.9	13.0	76.0	0.3
2-Monoglyceride	....	1.0	0.1	0.1	12.5	86.3	....
Tobacco (supplied as crude oil, triglycerides 89%, iodine value 138)							
Triglyceride	....	9.8	3.8	1.0	13.5	70.6	1.3
Plate (9 fns.)	0.1	11.2	4.6	0.6	14.1	68.5	0.9
2-Monoglyceride	....	0.9	0.3	0.2	15.8	82.2	0.6
Sunflower (Nigerian, extract 32%, triglycerides 99%, iodine value 125)							
Triglyceride	0.2	7.1	2.8	0.4	30.0	59.5	....
Columns (13 fns.)	....	8.0	3.5	0.5	32.7	55.3	....
2-Monoglyceride	....	1.1	0.3	....	25.0	73.6	....
Sunflower (Bulgarian, extract 31%, triglycerides 99%, iodine value 129)							
Triglyceride	....	7.0	4.4	0.3	26.0	62.3	....
2-Monoglyceride	....	1.1	0.3	....	23.9	74.7	....
Sunflower (Nigerian, extract 33%, triglycerides 99%, iodine value 112)							
Triglyceride	0.2	5.1	1.8	0.5	50.3	42.1	....
Columns (12 fns.)	....	5.4	2.4	0.4	52.5	39.3	....
2-Monoglyceride	....	1.0	0.2	....	49.9	48.9	....
***A. Mexicana* (extract 39%, triglycerides 87%, iodine value 117)**							
Triglyceride	....	12.3	4.2	0.3	28.1	55.1	....
Plate (9 fns.)	....	12.7	4.6	1.2	27.6	53.9	....
2-Monoglyceride	....	2.2	....	0.5	35.0	62.3	....
Maize (supplied as crude oil, triglycerides 93%, iodine value 120)							
Triglyceride	....	12.6	1.8	0.8	30.0	54.3	0.5
Plate (9 fns.)	....	14.3	2.7	1.1	29.0	51.9	1.0
2-Monoglyceride	....	2.4	....	0.3	29.1	68.2	....
Cotton (extract 20%, triglycerides 86%, iodine value 97)							
Triglyceride	1.1	27.3	3.1	1.4	16.7	50.4	....
Plate (9 fns.)	1.5	27.4	2.9	1.9	17.0	49.3	....
Plate (9 fns.)	1.7	26.6	4.5	2.7	16.7	47.8	....
2-Monoglyceride	0.3	3.6	0.3	0.7	24.5	70.6	....
Groundnut (extract 50%, triglycerides 91%, iodine value 80)							
Triglyceride[b]	....	9.8	3.7	0.4	60.9	18.1	....
Plate (9 fns.)[b]	....	10.5	3.8	0.6	59.9	18.5	....
2-Monoglyceride	....	1.8	0.2	0.4	66.6	31.0	....
***M. ternifolia* (extract 74%, triglycerides 94%, iodine value 75)**							
Triglyceride[c]	0.7	9.3	3.7	27.2	51.9	2.8	....
Plate (7 fns.)[b]	0.7	10.0	3.2	28.3	50.8	3.3	....
2-Monoglyceride	....	0.9	....	23.8	71.0	4.3	....
***M. ternifolia* (extract 68%, triglycerides 93%, iodine value 69)**							
Triglyceride[d]	0.5	10.1	6.2	18.3	55.4	3.4	....
2-Monoglyceride	....	0.8	....	15.5	77.6	6.1	....
***G. asiatica* (extract 60%, triglycerides 96%, iodine value 96)**							
Triglyceride[e]	....	10.1	8.1	0.3	28.8	37.6	....
Plate (12 fns.)[e]	....	10.0	7.5	0.5	29.4	39.7	....
2-Monoglyceride	....	1.6	....	0.9	37.3	60.2	....
***M. latifolia* (extract 46%, triglycerides 94%, iodine value 58)**							
Triglyceride	....	23.7	24.1	0.2	37.6	14.4	....
Plate (9 fns.)	....	22.9	25.8	0.7	37.3	13.3	....
2-Monoglyceride	....	3.1	4.5	....	60.4	32.0	....

[a] These figures refer to the number of carbon atoms and double bonds per molecule; thus 18:2 represents octadecadienoic acid.

	20:0	22:0	24:0	20:1	22:1
[b] also	1.4	2.7	1.4	1.3	0.3
	1.5	2.8	1.4	0.8	0.2
[c] also	2.4	....	....	2.0	....
	2.8	....	....	0.9	....
[d] also	3.7	....	....	2.4	....
[e] also	2.6	2.4	....	9.9	0.2
	2.6	1.5	....	8.3	0.5

Discussion

Efficiency of Thin-Layer Separation

By the thin-layer procedure we usually separate linoleic-containing oils into nine fractions in which individual glycerides, or groups of closely related glycerides, are separately concentrated. The effectiveness of this separation is apparent in Table II which shows the concentration of each glyceride or group of glycerides in the fraction in which it predominates. Most of these values exceed 80%, many exceed 90%, and lower values generally relate to minor glycerides (5% or below) which could only give high concentrations in smaller fractions than we choose to collect. We believe that if necessary these values could be increased and that chromatography on thin layers of silica containing silver nitrate provides an excellent method for the isolation and purification of glycerides.

In the separation of *Gmelina asiatica* glycerides, which contains about 10% of eicosenoic acid, there is evidence of subfractionation of monoethenoid C_{18} and C_{20} glycerides which was sufficient to distinguish between these two. This was less apparent in the monoethenoid C_{16} and C_{18} glycerides of *Macadamia ternifolia*.

The values quoted in Table I show that there is a reasonable agreement between the sum of the fractions recovered from the plate (or column) and the oil applied, though there is a tendency for the recovery of linoleic acid to be slightly low. The loss is smaller in the plate method than in the crystallisation-column method. We believe this loss arises mainly from the difficulty of completely extracting the more unsaturated glycerides from the silica-silver nitrate mixture.

TABLE II

Concentration (% mol) of Glycerides in Individual Fractions

	222	221	220	211	210	111	200	110	100[a]
Safflower	94	93	90	72	83	55	87	78	61
Tobacco	80	85	93	79	76	67	67	38	
A. mexicana	92	93	92	84	80	88	71	88	65
Maize	68	82	83	92	92	66	81	41	39
Cotton	74	65	92	57	95	....	87	65	89
Cotton	73	69	90	....	93	....	86	45	81
Groundnut	....	59	69	95	80	86	....	92	79
M. ternifolia	....	....	....	61	48	89	....	78	50
G asiatica	82	91	88	98	94	....	78	95	82
M. latifolia	....	....	....	50	75	40	....	52	94

[a] These figures indicate the number of double bonds in the three acyl chains. Each glyceride category includes all positional isomers.

Safflower and Tobacco

Results for these two oils, both of which contain over 70% of linoleic acid, are given in Table III along with results previously reported for oils of similar fatty acid composition. The high content of linoleic acid is reflected in the large proportion of glycerides containing three linoleic groups (47% and 33%) or two linoleic groups (37% and 43%). The values obtained by thin-layer chromatography agree with those calculated from lipolysis data or directly from the component acids according to a theory of positional distribution proposed by one of us (8). Our results are similar to those of Scholfield and Dutton (6) in their countercurrent distribution study of safflower oil; they do not show good agreement, particularly in respect of individual glycerides, with earlier results obtained by low temperature crystallisation (5,7). This is not surprising as this method is now recognised as unsuitable for oils containing appreciable quantities of more than one unsaturated acid.

Sunflower, Argemone mexicana, Maize, and Cotton

Our results for these oils are quoted, along with previous results where relevant, in Table III. We had three samples of sunflower seed oil, two of which possessed a similar fatty acid composition (Table I); all three samples were examined by lipolysis but only two by the longer crystallisation—chromatography procedure. Though only two of the three samples actually belong to this high linoleic group it is convenient to consider all three together.

Studies on sunflower glycerides, apart from the present work and that of Kaufmann (26), are confined to low temperature crystallisation studies and again there is poor agreement between the old and new results. The limitations of the early work have already been mentioned and our results show that a greater variety of glycerides is present in sunflower seed oil than was recognised before; thus three to five glyceride groups were differentiated by crystallisation, but eight by chromatography.

Our results for maize oil, though different from those obtained by low temperature crystallisation, resemble the incomplete analysis by countercurrent distribution (11) and results obtained by two different oxidation procedures (12,13). The component glycerides of *Argemone mexicana* have not been reported

TABLE III

Component Acids and Glycerides (% mol) of Seed Oils Containing Linoleic Acid

Reference	Safflower					Tobacco				Sunflower												*A. mexicana*		
	(5)	(6)	A[a]	B	C[c]	(7)	A[a]	B	C[c]	(5)	B[a]	C[c]	A[a]	B	C[c]	(26)	(5)	(5)	A[a]	B	C[c]	(9)	A[a]	B	C[c]
Component acids																									
16:0 + 18:0[c]	11	11		10		11		14		13	12			10		14	11	12		7		10		17	
16:1 + 18:1	13	13		13		17		14		24	26			30		28	33	44		51		49		28	
18:2 + 18:3	76	76		77		72		72		63	62			60		58	56	44		42		41		55	
Component glycerides																									
S_2U[d]	....	....	2	2	2	3	7	5	4	2	3	3	2	3	3	....	....	1	1	2	1	....	7	6	6
SU_2	32	....	26	26	26	27	33	32	33	35	28	28	26	25	25	....	32	34	22	18	19	31	38	37	37
U_3	68	....	72	72	72	70	62	63	63	63	79	79	72	72	72	....	68	65	77	80	80	69	55	57	57
322[f]	....	....	....	....	....	....	2	2	2	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
222	31	47	47	45	45	19	33	35	34	7	23	24	14	20	21	19	....	....	4	7	7	....	20	17	17
221	37	26	19	23	23	51	17	22	22	56	31	30	39	32	32	28	61	39	31	27	27	24	18	25	25
220	30	8	18	19	19	23	24	22	23	20	14	14	14	11	11	14	7	....	7	4	4	....	17	16	16
211	....	....	5	4	4	....	5	4	5	....	13	13	19	17	16	14	7	26	29	33	33	45	12	13	13
210	2	....	7	6	6	4	8	9	9	15	12	12	11	11	11	12	25	27	11	9	9	31	16	17	17
111	....	....	1	....	....	....	3	....	....	....	2	2	....	3	3	6	....	....	13	13	13	....	5	2	2
200	....	....	2	2	2	3	7	4	3	2	2	2	1	2	2		....	....	....	1	1	....	5	4	4
110	....	....	1	1	1	....	1	1	1	....	2	2	1	3	3	5	....	7	4	5	5	....	5	4	4
100	....	....	....	....	....	....		1	1	....	1	1	1	1	1	2	....	1	1	1	1	....	2	2	2
000	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....

Reference	Maize							Cottonseed										*M. latifolia*				
	A[a]	B	C[c]	(10)	(11)	(12)	(13)	A_1[a]	A_2[a]	B	C[c]	(13)	(14)	(15)	(16)	(17)	(18) A	(18) B[e]	A[a]	B	C[c]	(17)	(20)
Component acids																							
16:0 + 18:0[c]		14		15	13	13	16		32			33	29	27	27	23	....	....		48		43	44
16:1 + 18:1		31		24	27	27	!		18			17	22	16	!	!	....	....		38		!	43
18:2 + 18:3		55		61	60	60	!		50			50	47	57	!	!	....	....		14		!	13
Component glycerides																							
S_2U[d]	6	5	5	2	....	4	5	23	23	22	22	22	13	17	13	13	....	....	52	46	51	47	28
SU_2	38	33	34	41	....	34	34	50	52	48	50	51	59	48	48	44	....	....	39	41	41	36	71
U_3	56	62	61	57	....	62	61	27	25	29	28	27	28	35	39	43	....	....	8	9	8	17	....
322[f]	2	1	1	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
222	14	15	15	1	22	20	....	11	10	12	11	....	....	17	....	....	6	15	....	....	....	....	....
221	20	27	27	49	24	26	....	9	10	12	12	....	28(18)[b]	14	....	....	13	13	....	1	1	....	....
220	17	14	14	34	13	16	....	27	27	26	27	....	18(31)	29	....	....	30	26	3	2	3	....	....
211	14	16	15	7	....	14	....	5	3	4	4	....	....(9)	3	....	....			3	4	4	....	....
210	17	15	16	6	....	15	....	20	20	19	19	....	41(26)	17	....	....	23	18	15	17	16	....	....
111	6	3	3	....	....	2	....	2	2	1	1	....	....(1)	....	....	....			5	4	3	....	....
200	5	3	3	2	....	3	....	17	18	16	16	....	7(10)	12	....	....	23	21	17	15	14	....	....
110	4	4	4	1	....	3	....	3	5	3	4	....	....(2)	3	....	....			21	22	22	....	....
100	1	2	2	....	....	1	....	6	5	6	6	....	6(3)	5	....	....	5	7	35	31	37	....	....
000	....	....	....	....	....	....	....	....	....	1	....	....	....	....	....	....	....	....	1	4	....	....	....

[a] Present work.
[b] Result recalculated by Gunstone (ref. 8).
[c] See footnote, Table I.
[d] U and S refer to saturated and unsaturated acyl chains.
[e] Results obtained by chromatography (A), by lipolysis (B), and by direct calculation from the component acids (ref. 8,C).
[f] See footnote, Table II.

previously. They are very similar to those of maize oil, thus emphasising the previously accepted view that the glyceride composition of a seed oil depends on its component acids and not on its biological origin.

The component glycerides of cottonseed oil were first examined by Hilditch and Maddison (14) and their results have been reinterpreted and recalculated by Gunstone (8). More recently, Russian investigators (15) examined this oil by adsorption chromatography on alumina, by reverse phase chromatography, and by gas-liquid chromatography (GLC). The oil has also been examined by oxidation methods (13,16,17) and by lipolysis and thin-layer chromatography (TLC) (18). Bearing in mind slight differences in the composition of the oils examined, our results are similar to the more recent studies (only the Russian results are equally detailed) but differ from the earlier results, even after recalculation. Cottonseed oil was one of the first oils we examined by TLC and therefore we did the experiment twice. Both sets of results are given in Table II (A1 and A2); they show good agreement.

Groundnut and Macadamia ternifolia

These two oils are both rich in oleic acid but otherwise they have little resemblance. In groundnut oil (Table IV) four glycerides [211 (see footnote [a], Table II), 210, 111, and 110] make up almost 80% of the total but six minor glycerides are also present. The analysis by crystallisation picked out three major glycerides (211, 210, and 110; almost 80%) and three minor components also. Our results are similar to, but more detailed than, those of Barrett et al. (18), obtained by TLC and by lipolysis.

M. ternifolia seed oil is of interest in that it contains C_{16} and C_{18} monoethenoid acids in appreciable proportions but its component glycerides have not previously been reported. Our chromatographic study did not distinguish between glycerides in which these two acids were interchanged but the total amount in each group of similar glycerides agrees so well with those obtained by lipolysis that the figures for individual glycerides can be taken from the lipolysis results (column B, Table V). Our results show that five major glycerides (111, 11H, 1HH, 110 and H10) comprise over 80% of the oil and are accompanied by ten minor components.

Madhuca latifolia and Gmelina asiatica

Our sample of *G. asiatica* (Table IV) contained 23% of saturated acids along with 10% of eicosenoic acid which, like the saturated acids, is largely excluded from the 2-position. This content of "saturated" acids (33%) is interesting, for it is at this value that the difference between widest distribution (requiring 100% of S_2U) and positional distribution (requiring 50% of S_2U) is greatest (8). Our value of 48% is therefore highly significant. The subfrac-

TABLE IV

Component Acids and Glycerides (% mol) of Groundnut Oil and of *G. asiatica* Seed Oil

Ref.	Groundnut						*G. asiatica*		
	A[a]	B	C[a]	(18) A	(18) B[a]	(19) (17)	A[a]	B	C[a]
Component acids									
Sat.		19		....		20 20		23	
16:1 + 18:1[e]		61		....		59 ?		29	
20:1 + 22:1		2		....		21 ?		10	
18:2		18		....		.... ?		38	
Component glycerides									
S_2U[d]	11	10	9	....		7 9	21	24	25
SU_2	40	42	47	....		47 42	48	51	50
U_3	49	48	44	....		46 49	31	25	25
222[f]	....	....	1	....	....	....	6	4	5
221	5	5	6	8	3	6	10	10	10
22E[g]	....	....	....				6	5	5
211	4	2	2			....	12	11	11
21E	18	20	19				11	9	8
2EE	1	1	1	24	22	34	6	8	7
220	....	....	....				....	1	1
210	14	15	14			16	14	17	17
2E0	....	....	....				7	6	6
111	26	23	22	39	47		4	2	2
11E	....	2	2			6	3	3	3
1EE	....	....	....				....	1	1
200	4	3	2	....		....	7	7	7
110	21	22	24	26	24	31	7	7	7
E10	2	1	1				3	4	5
100	5	6	6	3	4	7	4	5	5

[a] Present work.
[c-f] Same as footnotes to Table III.
[g] E stands for eicosenoic acid and includes 20:1 and 22:1. In calculating the proportions of S_2U, etc., E is reckoned as a saturated acyl group (see text).

tionation of oleic and eicosenoic glycerides already referred to allowed us to distinguish between their glycerides with the results shown in Table IV. The greater number of acids in this oil means more glyceride categories. Fourteen are distinguished with four (221, 220, 211, and 210) exceeding 10% and a further six each present to the extent of 6 to 7%.

Mowrah butter (*M. latifolia*) with almost 50% of saturated acyl groups is the most saturated fat we examined (Table III). It contains eight categories of glycerides with four of them (210, 200, 110, and 110) comprising nearly 90% of the whole fat. The study of this more saturated material highlights one of the limitations of our chromatographic procedure in that it does not distinguish between the various saturated glycerides. Half of mowrah butter is 100 or 200 glycerides but within each group we cannot distinguish between the dipalmito-, the palmitostearo and the

TABLE V

Component Acids and Glycerides (% mol) of *M. ternifolia* Seed Oil

	A[a]	B	C[a]
Component acids			
Sat.		16	
16:1[e]		27	
18:1 + 20:1		54	
18:2		3	
Component glycerides			
S_2U[a]	5	6	6
SU_2	40	36	37
U_3	55	58	57
220[f]	1	....	....
211	4	2	2
21H[b]		2	2
2HH		1	1
210	4	2	2
2H0		1	1
111	51	15	15
11H		24	23
1HH		12	12
HHH		2	2
200	....	....	....
110	35	15	15
1H0		15	15
HH0		3	4
100	5	4	4
H00		2	2

[a] Same as footnote to Table III.
[e-f] Same as footnotes to Table III.
[b] H stands for hexadecenoic acid.

TABLE VI

Observed and Calculated[a] Proportions (% mol) of S_2U, SU_2 and U_3 Glycerides

Ref.		"Sat." acids[b] (% mol)	Observed S_2U	Observed SU_2	Observed U_3	Calculated S_2U	Calculated SU_2	Calculated U_3
3	Wild rose	5	1	15	84	1	13	86
*	Sunflower	7	1	22	77	1	19	80
3	Linseed	9	1	29	70	2	23	75
3	Candlenut	10	2	28	70	2	26	72
*	Sunflower	10	2	26	72	2	26	72
*	Safflower	10	2	26	72	2	26	72
12	Olive	12	5	33	62	3	30	67
12	Corn	13	4	34	62	4	31	65
*	Tobacco	14	7	33	62	4	34	62
2	*J. gossypifolia*	14	3	35	62	4	34	62
23	Poppy	14	0	32	68	4	34	62
*	Maize	14	6	38	56	4	34	62
16	Olive	15	6	35	59	5	35	60
*	*M. ternifolia*	16	5	40	55	6	36	58
3	Soya	16	6	38	56	6	36	58
16	Soya	17	6	38	56	7	37	56
*	*A. mexicana*	17	7	38	55	7	37	56
2	*J. curcas*	20	10	47	43	9	42	49
*	Groundnut	21	11	40	49	10	43	47
2	*J. multifida*	26	15	51	34	15	48	37
15	Cottonseed	27	17	48	35	16	49	35
*	Cottonseed	32	23	50	27	23	50	27
16	Cottonseed	33	21	49	29	25	50	25
*	*G. asiatica*	33	21	48	31	25	50	25
24	*Erythrina indica*	34	27	48	25	26	50	24
18	Shea	45	52	?	?	46	43	11
*	*M. latifolia*	48	52	39	8	52	40	8
18	Malayan palm	51	47	?	?	59	35	6
12	Cocoa butter	57	81	17	....	73	25	2
16	Cocoa butter	62	81	17	2	86	14	....
25	Cocoa butter	63	94	6	....	89	11	....
18	Cocoa butter	63	80	?	?	89	11	....

[a] Calculated according to Gunstone (8).
[b] 20:1 and 22:1 acids included with saturated acids.
* Present work.

disearo-monounsaturated glycerides. In this respect we consider our procedure to be complementary to that of Youngs et al. (13,16) which distinguishes between saturated but not between unsaturated acyl groups.

Chromatography, Lipolysis and Calculation of Component Glycerides from Component Acids

Results obtained in these three ways have been quoted in this paper and in two of our earlier papers (2,3). The agreement between values calculated from lipolysis data (column B) and those derived directly from component acids (column C) is to be expected and is not greatly significant since both are based on the same *assumption* that acyl groups present in the 2-position are associated statistically with those present in the 1- and 3-positions. They differ in that the lipolysis results allow for minor deviations from the mathematically limiting situation on which the theory is based (8).

The agreement between results obtained by our chromatographic procedures and those obtained by the other two methods is, on the other hand, much more meaningful for it provides support for the assumption adopted in handling lipolysis data and for the essential correctness of the postulates in the theory

TABLE VII

Proportions (% mol) of Polyethenoid Glycerides

	Component acids (% mol)		Component glycerides			
	Pe	X	Pe_3	Pe_2X	PeX_2	X_3[a]
Safflower	77	23	47	37	14	2
Tobacco	72	28	35	41	20	4
J. gossypifolia	69	31	33	48	16	3
Sunflower	60	40	14	53	31	2
A. mexicana	55	45	20	35	33	12
Maize	55	45	16	37	36	11
Cottonseed	50	50	11	36	42	11
J. multifida	49	51	9	38	43	10
Sunflower	42	58	4	38	40	18
G. asiatica	38	62	6	28	45	21
J. curcas	37	63	3	27	46	24
Groundnut	18	82	....	9	37	54
M. latifolia	14	86	....	3	35	62
M. ternifolia	3	97	....	1	8	91

[a] Pe and X refer to polyethenoid and other (monoethenoid and saturated) acyl chains.

TABLE VIII

Glyceride Categories (% mol) of Linoleic-Containing Oils

	"Sat." acids[a] (% mol)	Number of double bonds						
		7 and 6	5	4	3	2	1	0
Safflower	10	47	19	23	8	3	....	....
Tobacco	14	35	17	29	11	8	....	....
J. gossypifolia	14	33	22	32	9	3	1	....
Sunflower	10	14	39	33	11	2	1	....
Maize	14	16	20	31	23	9	1	....
A. mexicana	17	20	18	29	21	10	2	....
Sunflower	7	4	31	36	24	4	1	....
J. multifida	26	10	15	32	24	15	4	....
J. curcas	20	3	16	26	32	19	4	....
Cottonseed	32	11	9	32	22	20	6	....
G. asiatica	33	6	16	29	28	17	4	....
Groundnut	21	....	5	23	40	27	5	....
M. ternifolia	16	....	....	5	55	35	5	....
M. latifolia	48	....	....	6	20	38	35	1

[a] See footnote, Table VI.

of positional distribution. When first elaborated this theory was tested against results then available which were taken from Hilditch's monograph (21). Whilst agreement in the proportion of U_3, US_2, and S_3 glycerides was fair, agreement for U_2S glycerides was less satisfactory and this has been adversely commented upon (22). In Table VI we compare results for these categories of glycerides calculated on the basis of our theory of positional distribution with recent results obtained by ourselves or by others. These show a much better agreement than the earlier results. It should be noted that these comments apply only to vegetable fats.

Polyethenoid Glycerides

In Tables VII and VIII results obtained from our linoleic-containing oils are classified according to the number of polyethenoid acid groups present in the glyceride and the number of double bonds present in the glycerides. The safflower, tobacco, *Jatropha glandulifera,* and the linoleic-rich sunflower seed oil, each with more than 60% of linoleic acid, contain 67–84% of glycerides having two or three linoleic chains and are the only oils listed here which are likely to show drying properties.

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Glyceride Studies. V. The Distribution of Unsaturated Acyl Groups in Vegetable Triglycerides

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Abstract

The distribution of oleic, linoleic, linolenic, petroselinic, hexadec-9 and 11-enoic, sterculic, four conjugated octadecatrienoic acids, isolinolenic, and octadeca-6,9,12,15-tetraenoic acid in vegetable triglycerides has been studied by hydrolysis with pancreatic lipase. The results, discussed in terms of a selectivity factor, indicate that these unsaturated acids do not compete equally for the secondary hydroxyl group.

Introduction

PANCREATIC LIPASE is known to remove acyl groups attached to the two primary glycerol hydroxyls in preference to those attached to the secondary hydroxyl group and lipolysis of vegetable fats by several investigators (1–6) has shown that in most cases 95–100% of the fatty acids in the 2-position are unsaturated C_{18} acids (oleic, linoleic, and linolenic) even when the total content of these acids is as low as 37 or 38% (see reference 7 for a summary of results). This important result has emphasised the non-random character of acyl group distribution in vegetable glycerides and has led to the wide acceptance of the theory of positional distribution (7–9) in place of the earlier ideas of random and widest distribution, neither of which is entirely acceptable. Gunstone (7) and Mattson and Volpenhein (5) have suggested that the acids found in natural triglycerides fall into two groups: those which are preferentially esterified at the 1- and 3- positions (designated "saturated" by Gunstone and Category I acids by Mattson and Volpenhein) and those which are preferentially esterified at the 2-position ("unsaturated" or Category II acids). Mattson and Volpenhein (4) had earlier shown that though oleic, linoleic, and linolenic acids belong to Category II, the C_{20} and C_{22} monoethenoid acids, which characterise the Cruciferae, belong to Category I, behaving like palmitic and stearic acids. From an examination of the distribution of the three unsaturated C_{18} acids these same authors (5) conclude that there is a slight tendency for there to be more linoleic and less oleic in the 2-position than would be expected from their pro-

TABLE I

Enrichment and Selectivity Factors for Acids in Linseed Oil and Morning Glory Seed Oil (4)

	16:0[a]	18:0	16:1	18:1	18:2	18:3	Unsat. acids
Linseed							
Triglyceride (% mol.)	6.1	3.2	0.1	16.6	14.2	59.8	90.7
2-Monoglyceride (% mol.)	0.7	—	0.2	18.8	20.6	59.7	99.3
Enrichment factor	0.11	—	—	1.13	1.45	1.00	1.09
Selectivity factor	—	—	—	1.04	1.33	0.92	—
Morning glory							
Triglyceride (% mol.)	22	12	—	15	42	8	65
2-Monoglyceride (% mol.)	2	1	—	23	64	10	97
Enrichment factor	0.09	0.08	—	1.53	1.52	1.25	1.49
Selectivity factor	—	—	—	1.03	1.02	0.84	—

[a] These figures indicate the number of carbon atoms and double bonds per acid molecule; thus 18:2 represents octadecadienoic acid.

portion in the triglyceride but that otherwise "each of the acids in Category II is randomly distributed among the positions in the triglyceride molecules that are not occupied by Category I acids." We consider the consistent preference of linoleic over oleic acid for the 2-position as significant and we have extended this type of observation to a wider range of unsaturated acids.

Previously we used the concept of an *enrichment factor* (10,11) to discuss acyl group distribution. This is useful when comparing values for acids competing for the 2-position in the same fat, it is less convenient for discussing the behaviour of acids in several different fats and for this reason we now use another term: the *selectivity factor*. The enrichment factor is the ratio of the concentration (molar) of an acid group in the 2-position to its concentration in the total triglyceride. The selectivity factor is the enrichment factor of a particular acid divided by the enrichment factor for all the Category II acids present in the fat under consideration.

This is illustrated by the values for linseed oil detailed in Table I. Enrichment factors are obtained for each acid by dividing the value in the second row of figures by that in the first row. The selectivity factors of the three unsaturated acids result when the individual enrichment factors are divided by the enrichment factor for the unsaturated C_{18} acids taken as a group (1.09). In general we quote enrichment and selectivity factors to one place of decimals; this value may not be very accurate when the proportion of an acid falls below 5% in the triglycerides.

Table I also contains the enrichment and selectivity factors for the same three unsaturated C_{18} acids in Morning Glory seed oil which contains 35% of Category I acids compared with only 9% in linseed oil. The two sets of enrichment factors illustrate the difficulty in comparing these factors in oils with very different proportions of Category I acids. The selectivity factor compensates for these differences and makes

TABLE II

Component Acids (% mol.) of Triglycerides and 2-Monoglycerides

(i) *Gevuina avellana*	16:0	18:0	20:0	22:0	16:1	18:1	18:2	20:1	22:1[a]
1st sample									
Triglyceride	2.3	0.6	1.4	2.0	24.0	43.7	7.8	9.9	8.3
2-Monoglyceride	0.5	—	—	—	26.6	57.3	15.6	—	—
2nd sample									
Triglyceride	2.2	0.7	1.5	1.9	23.9	41.8	8.8	10.3	8.9
2-Monoglyceride	0.4	—	—	—	26.3	54.7	18.6	—	—

(ii) Seed oils containing conjugated trienoic acids	16:0	18:0	18:1	18:2	18:3 (conj.)	Position of unsaturation
Tung oil						
Triglyceride	3.1	2.1	11.2	14.6	69.0	9c, 11t, 13t
2-Monoglyceride	0.3	—	8.8	30.5	60.4	
Momordica balsamina						
Triglyceride	9.6	5.8	7.4	9.7	68.0	9c, 11t, 13c
2-Monoglyceride	1.0	—	6.0	20.7	72.3	
Catalpa bignonoides						
Triglyceride	2.3	1.5	9.6	45.1	41.5	9t, 11t, 13c
2-Monoglyceride	0.4	—	14.8	76.2	8.6	
Calendula officinalis						
Triglyceride	3.0	—	4.3	28.8	63.9	8t, 10t, 12c
2-Monoglyceride	0.7	—	4.6	14.4	80.3	

(iii) Seed oils containing isolinolenic and octadecatetraenoic acids	16:0	18:0	16:1	18:1	18:2	18:3	iso 18:3	18:4	20:1
Oenothera crocata									
Trigilyceride	4.3	1.3	1.0	75.6	17.8	—	Tr	—	—
2-Monoglyceride	1.7	—	1.1	77.1	16.5	—	3.6	—	—
Oenothera tetraptera									
Triglyceride	14.7	2.0	Tr	5.2	77.4	—	0.7	—	—
2-Monoglyceride	1.6	0.3	0.4	6.9	90.8	—	Tr	—	—
Oenothera biennis									
Triglyceride	9.2	2.3	0.7	12.0	65.8	—	10.0	—	—
2-Monoglyceride	—	0.7	—	15.7	68.1	—	15.5	—	—
Oenothera macrocarpa									
Triglyceride	7.6	1.7	0.5	12.4	66.7	—	11.1	—	—
2-Monoglyceride	—	—	—	15.0	64.5	—	20.5	—	—
Oenothera missouriensis									
Triglyceride	6.5	1.7	—	13.0	65.1	Tr	11.7	—	—
2-Monoglyceride	3.2	1.4	—	21.1	53.6	2.3	18.4	—	—
Forget-me-not									
Triglyceride	11.2	2.0	0.4	33.0	27.6	4.1	14.6	(3.0)	(4.1)
2-Monoglyceride	0.7	—	—	23.1	27.8	4.8	38.0	5.6	—
Blue bur									
Triglyceride	5.9	1.9	0.3	12.8	14.8	35.9	8.7	(17.7)	(2.0)
2-Monoglyceride	0.5	—	0.4	10.7	15.7	27.0	16.9	28.8	—
Borage									
Triglyceride	11.8	4.1	0.3	17.4	37.8	—	21.6	—	4.0[b]
2-Monoglyceride	0.6	—	—	13.7	37.5	—	48.2	—	—
								iso	
Anchusa azurea									
Triglyceride	8.2	1.5	0.8	23.1	32.5	18.4	10.8	(2.5)	(2.2)
2-Monoglyceride	0.7	—	—	16.7	51.9	6.7	17.5	6.5	—

(iv) *Bombacopsis glabra*	16:0	18:0	18:1	18:2	Malvalic	Sterculic	Dihydro-sterculic
Triglyceride	53.3	2.7	7.6	4.2	1.6	27.4	3.2
2-Monoglyceride	5.4	—	16.2	10.7	1.3	62.1	4.3

[a] See footnote Table I.
[b] Also 22:0, 0.9%; 22:1, 2.1%.

comparison between the two oils easier and more meaningful.

Procedure

We shall discuss results obtained by other workers along with those which we have reported in earlier papers (11–13). Details of experimental procedure are given in these papers but brief notes are added concerning the oils not previously reported. Results are summarised in Table II.

The component acids of the petroselinic acid-containing oils were examined by a new method which will be fully described elsewhere. Oleic and petroselinic esters are not adequately separated by gas liquid chromatography and the proportion of these is determined from the relative amounts of lauric acid (from the Δ^6 isomer) and azelaic acid (from the Δ^9 isomer) produced after oxidation. The results may be slightly less accurate than those obtained in the usual type of fatty acid mixture but are better than earlier results based on the differing solubilities of the lead salts of these two isomeric acids.

Two samples of *Gevuina avellana* were examined. Both contain about 40% of oil and gave about 90% of neutral triglycerides. Our results are quantitatively similar to those previously reported by Cattaneo *et al.* (14) and we have confirmed their observation that the unusual unsaturated acid is hexadec-11-enoic acid. This acid, isolated by a combination of preparative gas liquid chromatography and column chromatography on silica-silver nitrate, gave undecanedioic acid as the only dibasic acid after periodate-permanganate oxidation (15).

When chromatographing esters from oils containing conjugated trienoic acids we observed a tendency for the conjugated esters to appear as two overlapping peaks, perhaps because of isomerisation on the hot chromatographic column (16). Despite this, the combined areas of these two peaks gave a good assessment of the proportion of conjugated esters when checked against the proportion determined by ultraviolet spectroscopy.

Oils containing isolinolenic acid (octadeca-6,9,12-trienoic) present no difficulty because the ester is well resolved from both linoleate and linolenate on a polyester column. The esters of octadecatetraenoic acid and eicosenoic acid, however, overlap on polyester columns and oils containing these acids must also be examined on non-polar (Apiezon L) columns (17). Figures given for these two acids may be slightly less accurate than most of the other values.

Chromatographic analysis of *Bombacopsis glabra* (18) presents difficulties because of the instability of cyclopropene esters (18,19). We isolated the oil with petroleum ether (b.p. 40–60C) at room temperature and prepared the methyl esters by transesterification with sodium methoxide in boiling methanol for five minutes only (20). The esters were examined by gas liquid chromatography on Apiezon L and polyethylene glycol adipate columns before and after hydrogenation in methanol solution in presence of 5% palladium-charcoal.

Discussion

Oleic and Linoleic Acids

Information about 49 seed oils containing oleic and linoleic acid as the only significant unsaturated acids is contained in Table III. These results confirm and extend the views of Mattson and Volpenhein (5). Selectivity factors greater than 1.0 correspond to points lying *above* the "theory" lines drawn in their figures, factors below 1.0 correspond to points *below* the "theory" lines. As the summary at the end of Table III shows linoleic acid almost always has a selectivity factor greater than oleic acid so that the chance of linoleic acid being present in the 2-position is slightly higher and that of oleic acid slightly lower than expected.

TABLE III

Selectivity Factors for Oils Containing Oleic and Linoleic Acid

Ref.	Name	Component Acids (% mol.) "sat."	18:1	18:2[a]	Selectivity Factors 18:1	18:2
13	Sunflower	7	50	42	0.9	1.1
5	Filbert	9	82	8	1.0	1.4
5	Almond	9	70	21	0.8	1.5
5	Sunflower	9	27	64	0.8	1.1
5	Onion	10	26	64	0.9	1.0
13	Safflower	10	13	77	0.9	1.0
13	Sunflower	10	30	60	0.8	1.1
5	Acorn	10	69	20	0.9	1.4
5	Safflower	11	14	75	0.8	1.0
13	Sunflower	12	26	62	0.8	1.1
5	Pecan	12	58	30	0.9	1.2
5	Poppy	12	11	76	0.7	1.0
5	Spinach	13	24	60	0.8	1.1
13	Tobacco	14	14	71	1.0	1.0
11	*Jatropha gossypifolia*	14	17	69	1.0	1.0
5	Corn	14	29	58	0.8	1.1
5	Olive	14	76	8	1.0	1.1
13	Maize	15	30	54	0.9	1.1
1	Olive	15	74	10	1.0	1.0
3	Olive	15	74	11	1.0	0.8
5	Tomato	16	21	61	0.9	1.0
5	Sesame	16	40	44	0.9	1.1
13	*Argemone mexicana*	17	28	55	1.1	1.0
4	Peanut	18	52	27	0.8	1.4
5	Rice	19	41	38	0.9	1.2
13	Groundnut	19	61	18	0.9	1.4
11	*Jatropha curcas*	20	40	37	0.8	1.2
5	Cucumber	21	7	71	0.5	1.1
1	Peanut	21	50	29	0.9	1.2
5	Cashew	22	60	18	0.7	1.9
4	Morro	24	51	22	0.9	1.2
5	Squash	24	16	60	0.8	1.1
11	*Jatropha multifida*	26	23	49	0.8	1.1
4	Marigold	28	9	62	0.8	1.0
5	Cottonseed	28	17	55	0.9	1.0
5	Brazil nut	29	33	39	0.9	1.1
5	Cottonseed	30	17	52	0.9	1.0
1	Cottonseed	30	19	51	0.8	1.1
13	Cottonseed	32	18	50	1.1	1.0
2	Illipé	42	53	5	1.0	0.7
4	Coffee	44	7	48	0.8	1.0
6	Shea	46	47	7	1.0	1.3
2	Palm	46	46	8	1.0	1.2
13	*Madhuca latifolia*	48	38	14	0.9	1.3
1	Palm	49	40	11	1.0	0.9
5	Palm	50	39	9	1.0	1.4
2	Karité	50	45	5	1.0	1.4
6	Palm	51	42	7	1.0	1.1
1	Shea	52	41	6	1.0	1.3

	Selectivity factor <1.0	1.0	>1.0	The larger value
Oleic	32	15	2	5 times
Linoleic	3	14	32	41 times

[a] See footnote, Table I; minor amounts of 16:1 and 18:3 are neglected.

TABLE IV

Selectivity Factors for Oils Containing Linolenic Acid

Ref.	Name	Component acids (% mol.) Sat.	18:1	18:2	18:3	20:1+ 22:1[a]	Selectivity factors 18:1	18:2	18:3
12	Wild rose	4	11	49	36	—	1.0	1.1	0.9
5	Wheat flour	8	27	59	7	—	1.2	1.1	0.8
12	Linseed	9	17	14	60	—	1.0	1.3	0.9
5	Linseed	10	22	15	52	—	1.1	1.4	0.8
12	Candlenut	10	22	37	31	—	1.1	1.3	0.6
5	Walnut	11	15	61	12	—	1.0	1.0	0.9
1	Soya	13	27	52	8	—	1.4	0.9	0.7
5	Soya	16	25	51	8	—	0.7	1.1	0.9
12	Soya	16	24	51	9	—	0.8	1.1	0.8
5	Wheat germ	20	18	55	7	—	0.8	1.1	0.7
4	Morning glory	35	15	42	8	—	1.0	1.0	0.8
4	Mustard	3	16	10	14	56	1.1	1.1	0.9
4	Turnip	4	15	14	12	54	0.9	1.1	1.0
4	Kale	5	22	12	6	54	0.9	1.0	1.2
4	Rape	5	17	17	11	50	0.9	1.1	0.9
4	Rape	6	22	15	14	43	0.9	1.3	0.9
4	Cabbage	7	16	17	12	48	0.8	1.1	1.1
4	*Erysimum perotskianum*	8	13	27	33	28	1.0	1.2	0.9
4	Radish	11	22	15	12	39	0.8	1.2	1.2
4	*Descurania sophia*	12	13	18	38	19	0.8	1.1	1.0

	Selectivity factor <1.0	1.0	>1.0	The largest value
Oleic	10	5	5	Twice
Linoleic	1	3	16	12 times
Linolenic	15	2	3	Once

[a] See footnote, Table I.

TABLE V

Selectivity Factors for Oils Containing Petroselinic Acid

Name	Component acids (% mol.) Sat.	18:1(6)	18:1(9)	18:2[a]	Selectivity factors 18:1(6)	18:1(9)	18:2
Parsley	3	82	4	10	1.0	—	0.9
Carrot	5	73	9	13	0.8	1.5	1.7
Parsnip	5	60	10	24	0.6	1.8	1.8
Caraway	6	47	13	34	1.1	1.2	0.9
Chervil	9	53	3	34	1.6	—	0.2

[a] See footnote, Table I: the figure in parentheses shows the double bond position.

Linolenic Acid

Linolenic acid-containing oils examined by lipolysis fall into two groups. Eleven oils having only C_{18} unsaturated acids are listed in the upper part of Table IV and nine oils in which these unsaturated acids are accompanied by C_{20} and C_{22} monoethenoid acids are listed in the lower part. These longer-chain acids, though unsaturated, behave as Category I acids and have been treated as such in calculating selectivity factors.

In both groups of seed fats linoleic consistently shows a high selectivity factor; 16 out of 20 values exceed 1.0 and only one value is below 1.0. The behaviour of oleic and linolenic acid differs slightly in the two groups of oils. In all the oils having only C_{18}-unsaturated acids, linolenic acid has a selectivity factor below 1.0 (markedly below in most cases), whereas oleic acid shows rather more affinity for the 2-position and has a selectivity factor of 1.0 or more in eight oils, though in most of these it still has a value below that of linoleic acid. In the oils also containing C_{20} and C_{22} acids, linolenic acid shows slightly more affinity for the 2-position competing about equally with oleic acid, but still less effectively than linoleic acid. It seems that in the biosynthesis of vegetable triglycerides linoleic acid is slightly more effective in acylating the secondary hydroxyl group than either oleic or linolenic acid.

Petroselinic Acid

Petroselinic acid (octadec-6-enoic) is an isomer of oleic acid characteristic of the Umbelliferae where it occurs along with oleic and linoleic acids and minor amounts of saturated acids. Our lipolysis studies (Table V) show that petroselinic acid accompanies oleic and linoleic in the 2-position but the selectivity factors for the three unsaturated acids in the five oils examined show rather more variation than in the linoleic and linolenic containing oils. Chervil seed oil seems to be unusual in that its linoleic acid shows a remarkably low selectivity factor and we have confirmed that the octadecadienoic acid is the 9*c*, 12*c* isomer.

Hexadecenoic Acids

Though hexadecenoic acid rarely exceeds 1 or 2% of the component acids, it is a major component of a few seed oils. The results for avocado have previously been given by Mattson and Volpenhein (5) and we have now examined two samples of *Macadamia ternifolia* seed oil and two of *Gevuina avellana* seed oil with the results given in Table VI. The behaviour of oleic and linoleic acid is normal in that oleic has a selectivity factor close to 1.0 and linoleic has a higher factor. Hexadecenoic acid, whether the Δ^9 acid in avocado or *M. ternifolia* or the Δ^{11} acid in *G. avellana*, occurs in the 2-position but its low selectivity factor shows that it is less likely to be found there than either of the C_{18} unsaturated acids which accompany it.

TABLE VI

Selectivity Factors for Oils Containing Hexadecenoic Acid

Ref.	Name	Component acids (% mol.) "Sat."	16:1	18:1	18:2[a]	Selectivity factors 16:1	18:1	18:2
5	Avocado	37	20	27	14	0.4	1.0	1.8
13	*Macadamia ternifolia*	18	27	52	3	0.7	1.1	—
		23	18	56	3	0.7	1.1	—
*	*Gevuina avellana*	24	24	44	8	0.8	1.0	1.5
		25	24	42	9	0.8	1.0	1.6

[a] See footnote, Table 1.
* Present work.

TABLE VII

Selectivity Factors for Oils Containing Conjugated C_{18}-Trienoic Acids

		Component acids (% mol.) Sat.	18:1	18:2	conj. 18:3[a]	Selectivity factors 18:1	18:2	conj. 18:3
Tung	9*c*, 11*t*, 13*t*	5	11	15	69	0.8	2.0	0.8
Momordica balsamina	9*c*, 11*t*, 13*c*	15	7	10	68	0.7	1.8	0.9
Catalpa bignonoides	9*t*, 11*t*, 13*c*	4	10	45	41	0.9	1.0	—[b]
Calendula officinalis	8*t*, 10*t*, 12*c*	3	4	29	64	1.1	0.5	1.2

[a] See footnote, Table 1.
[b] Considered to be a Category I acid (see text).

Conjugated Octadecatrienoic Acids

Elaeostearic acid (9*c*, 11*t*, 13*t*) is the best known of the C_{18} conjugated trienoic acids but recent work, particularly by Hopkins, has shown the occurrence of several configurational and positional isomers. We have been able to examine three of these in addition to elaeostearic acid: the 9*c*, 11*t*, 13*c* isomer in *Momordica balsamina* (21), the 9*t*, 11*t*, 13*c* isomer in *Catalpa bignonoides* (22), and the 8*t*, 10*t*, 12*c* isomer in *Calendula officinalis* (23).

These four isomeric acids show remarkable differences in their selectivity factors. Tung oil and *M. balsamina* seed oil, though containing different acids, are fairly similar; the conjugated triene acid acylates the secondary hydroxyl with about the same efficiency as oleic acid but much less effectively than linoleic acid which shows very high factors (2.0 and 1.8). The triene acid (catalpic) in *C. bignonoides* (9*t*, 11*t*, 13*c*), on the other hand, behaves as a Category I acid. The triglycerides contain 42% of this acid but the 2-monoglyceride contains only 9% and the enrichment factor (0.2) is of the same order as is commonly attained by palmitic acid in many seed fats. The selectivity factors for oleic and linoleic acid (Table VII) are therefore calculated on the basis that the trienoic acid belongs to Category I. *C. officinalis* seed oil, containing a C_{18} acid with the same configuration as catalpic but in the 8, 10, 12 rather than the 9, 11, 13 positions is different again. The conjugated acid and oleic acid have high selectivity factors whilst linoleic has an unusually low value. Because of this we isolated the C_{18}-dienoic acid and proved it to be the 9*c*, 12*c* isomer. Another illustration of the unusual behaviour of these conjugated C_{18} trienoic acids is found in the lipolytic behaviour of *M. charantia* seed oil (24); glyceride composition calculated from lipolysis results does not agree with that obtained by Young's oxidation procedure.

Isolinolenic and Octadecatetraenoic Acids

The *Oenothera* genus is unusual in that the seed fats of some, but not all, species contain isolinolenic acid (octadeca-6,9,12-trienoic); we found this acid in three of the five species examined. Some Boraginaceae species have recently been shown (17,25,26) to contain in their seed oils, in addition to oleic and linoleic acid,

TABLE VIII

Selectivity Factors for Oils Containing Isolinolenic and Octadecatetraenoic Acids

	Component acids (% mol.)						Selectivity factors				
	"Sat."	18:1	18:2	18:3	iso 18:3	18:4[a]	18:1	18:2	18:3	iso 18:3	18:4
Oenothera crocata	6	76	18	—	—	—	1.0	0.9	—	—	—
O. tetraptera	17	5	77	—	1	—	1.2	1.0	—	—	—
O. biennis	12	12	66	—	10	—	1.2	0.9	—	1.4	—
O. macrocarpa	9	12	67	—	11	—	1.1	0.9	—	1.7	—
O. missouriensis	10	13	65	—	12	—	1.5	0.8	—	1.5	—
Forget-me-not	17	33	28	4	15	3	0.6	0.8	—	2.1	(1.7)
Blue bur	10	13	15	35	9	18	0.8	1.0	0.7	1.7	(1.5)
Borage	23	17	38	—	22	—	0.6	0.8	—	1.7	—
Anchusa azurea	12	23	32	18	11	3	0.8	1.4	0.6	1.4	(2.1)

[a] See footnote, Table 1.

linolenic acid and/or isolinolenic acid and/or octadeca-6,9,12,15-tetraenoic acid and we have been able to examine four of these seed oils.

The results from the *Oenothera* oils are unusual in that oleic acid has, surprisingly and consistently, a higher selectivity factor than linoleic acid and, even more interesting, isolinolenic acid shows a very high selectivity factor. In the Boraginaceae oleic and linoleic fall into their more customary order though the values tend to be lower than usual. In contrast, the factors for isolinolenic acid and (less certainly) for octadecatetraenoic acid are unusually high. The seven selectivity factors for isolinolenic acid lie between 1.4 and 2.0. This means that the proportion of this acid in the 2-position is 40–100% higher than would be expected if all Category II acids were statistically distributed at this position. This high value is in marked contrast to the low factor associated with the more common isomeric form of linolenic acid.

Sterculic Acid

Based on a single result for *Bombacopsis glabra* seed oil sterculic acid [8-(2′-*n*-octylcycloprop-1′-enyl)-octanoic] seems to take its place along with oleic and linoleic acid in the 2-position. However it should be noted that oleic and linoleic acid comprise only 12% of the total acids and cannot therefore fill all the 2-position, and that the total content of unsaturated acids is only 41%.

General Comments

Our results must be considered only as a preliminary communication. They provide interesting evidence about the distribution of acyl groups between the secondary and the two primary hydroxyl groups of glycerol but they need to be supported by more information before any very definite conclusions are obtained. We plan to do this in two directions: we hope to examine more seed oils containing the acids we have discussed in this paper and we hope to extend the range of acids. In view of the size of this task and the difficulty of obtaining samples we hope that others will also direct their attention to this problem and that lipolysis data can be expressed in similar terms to ours.

Obviously it is inadequate to think of acids as merely falling into two groups each member of which behaves in identical manner. It has been known for some time that Category I acids acylate the 2-position to a minor extent and do so entirely when Category II acids are absent or present in very small amount. It is now apparent that Category II acids vary in their ability to acylate the 2-position; some tend to get more than their statistical share of the secondary hydroxyl groups, others get rather less. The acids which have been examined so far can be listed in the following order:

	Selectivity factor
Category II	
(i) Isolinolenic, octadecatetraenoic	1.4–2.0
(ii) Linoleic, 18:3(8*t*, 10*t*, 12*c*)	1.0–1.2
(iii) Oleic, petroselinic, linolenic, sterculic 18:3 (9*c*, 11*t*, 13*t*), 18:3(9*c*, 11*t*, 13*c*)	0.8–1.0
(iv) Hexadecenoic	0.7–0.8
Category I	Enrichment factor
Eicosenoic, docosenoic, palmitic, stearic, 18:3 (9*t*, 11*t*, 13*c*)	>0.2

This must be considered as a provisional sequence which may need considerable modification as further results become available. It is difficult at this stage to see a pattern in these results, especially in the widely differing results obtained with the conjugated trienoic acids, but it is interesting that two of the acids in group (i) and (ii) are essential fatty acids (linoleic and isolinolenic) and that the conjugated trienoic acid also contains the end group, $CH_3 \cdot [CH_2]_4 \cdot CH =$ (*cis*), characteristic of the linoleic family.

Finally it must be acknowledged that these results depend on the reliability of the enzymatic hydrolysis procedure. There is plenty of evidence that for the *normal range of saturated and unsaturated acids* pancreatic lipase is primarily position-specific (showing a strong preference for the 1- and 3- positions) and only to a very minor extent structure-specific, i.e. depending on the structure of the glyceridic acyl group. It is not possible to say with certainty that this behaviour will extend to the less common unsaturated acids and further work is required to check this.

TABLE IX

Selectivity Factor for an Oil Containing Sterculic Acid

	Component acids (% mol)				Selectivity factors		
	"Sat."	18:1	18:2[a]	sterculic	18:1	18:2	sterculic
B. glabra	56	8	4	27	1.0	1.2	1.1

[a] See footnote, Table 1.

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